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Estimation of Nucleic Acids in Tissue from the Nervous System

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The starting point of this investigation was the report of Davidson & Waymouth (1944) that white matter of the brain of the sheep contained a higher concentration of protein-bound phosphorus, i.e. phosphorus remaining after removal of lipid phosphorus and acid-soluble phosphorus, than grey matter. If most of this protein-bound phosphorus were present as nucleic acid, there remained the possibility that nucleic acid might be an important constituent of the myelin sheath of a mammalian nerve fibre. Since no nucleic acid can be detected in the myelin sheath by the usual histochemical means, it seemed worth while to investigate the matter further. Attempts were made to estimate the concentration of deoxypentosenucleic acid (DNA) and pentosenucleic acid (PNA) in different parts of the nervous system by two methods at present widely used, that of Schmidt & Thannhauser (1945) and that of Schneider (1945). Although recovery experiments showed that added DNA, PNA, or mixtures of the two, could be recovered quantitatively from brain extracts by either method, it soon became apparent that the two methods gave widely diverging results when they were applied to white matter and grey matter of brain and to peripheral nerve.

It was decided, therefore to investigate the validity of these methods for tissue from the nervous system. The conflicting results are due, in part, to the presence of considerable amounts of a phosphorus-containing compound in brain and spinal cord, particularly in the myelin-rich white matter. The phosphorus of this compound is not removed by ice-cold 10% (w/v) trichloroacetic acid (TCA) nor is it removed by the usual ethanol-ether treatment for the extraction of lipids. This substance is probably the same as the inositol-containing

trypsin-resistant lipid-protein complex isolated from white matter by Folch & Le Baron (1951). Dr J. Folch, McLean Hospital, Waverley, Mass., in experiments as yet unpublished, has obtained results similar to those reported here.

In addition, it is shown that TCA extracts of brain and nerve tissue, prepared as described by Schneider (1945), contain chromogenic material that interferes with the colour reactions of Dische (1930) for DNA and Meibbaum (1939) for PNA. Folch (1951) suggests that this interference may be caused by traces of the complex lipid substance, strandin, isolated from brain by Folch, Arsove & Meath (1951). A preliminary account of these experiments has already appeared (Rossiter, Logan & Mannell, 1951).

METHODS

Standards

The same DNA and PNA standards were employed throughout this work. Results obtained for each of the methods are comparable inasmuch as they are all given in terms of the P content of these standards. The DNA standard was a preparation of calf sodium thymonucleate prepared by the

Table 1. *Absorption characteristics and phosphorus content of standard preparations of DNA and PNA*

	$\lambda_{\text{max.}}$ (m μ .)	ϵ (P) at $\lambda_{\text{max.}}$	$\lambda_{\text{max.}}$ (m μ .)	ϵ (P) at $\lambda_{\text{min.}}$	P (%)
DNA	259	7300	230	2900	8.3
PNA	258	9300	228	3500	8.5

method of Mirsky & Pollister (1942), and kindly provided by Dr G. C. Butler, Department of Biochemistry, University of Toronto. The PNA standard was a preparation of yeast sodium ribonucleate selected from a number of commercial preparations examined. Table 1 shows that the absorption

characteristics and P content of the standards are in good agreement with the published figures for DNA and PNA (Stimson & Reuter, 1945; Di Carlo & Schultz, 1948; Blout & Fields, 1949; Chargaff *et al.* 1949, 1950).

The symbol $\epsilon(P)$ is used for the atomic extinction coefficient with respect to P as defined by Chargaff & Zamenhof (1948), i.e. $\epsilon(P) = \frac{30 \cdot 98 E}{cl}$, where E is the extinction ($\log I_0/I$), c is the concentration of P in g./l. and l is the thickness of the absorbing layer in cm. The value of $\epsilon(P)$ at $\lambda_{\max}$ is a little greater than that given by Chargaff *et al.* (1949). The value of $\epsilon(P)$ probably depends upon the degree of polymerization of the sample (Chargaff *et al.* 1950). For samples of thymus DNA, presumably less highly polymerized, Stimson & Reuter (1945) and Blout & Fields (1949) reported much higher values of $\epsilon(P)$.

matter was chiefly corpus callosum. The sciatic nerve of the cats was rapidly cleaned of adherent fatty and epineural connective tissue and the whole nerve frozen and ground to a powder as described for the dog brain.

Analytical methods

For each tissue two pairs of duplicate samples of the frozen powder (100–200 mg.) were transferred to tared centrifuge tubes and the tubes reweighed. After the removal of acid-soluble P (I) and lipid P (II), as described by Schneider (1945), each pair of duplicate samples was submitted to either the Schmidt-Thannhauser or Schneider procedure as outlined in Table 2. One pair was hydrolysed for 18 hr. at 37° in N -KOH. The hydrolysate was divided into two portions, one of which was used for the determination of total P (III) and the other for the precipitation of the

Table 2. Scheme outlining determinations

Preparation of tissue

Estimations were done on the white matter and grey matter of the brains of six dogs and on the sciatic nerves of six cats. The animals were anaesthetized with nembutal, bled, and the brain or sciatic nerve removed as soon as possible after death. Samples of brain tissue (0.5–1 g.) were frozen in liquid N_2 and ground to a fine powder in a mortar. The sample of grey matter consisted of thin shavings of cortex from the cerebral hemispheres. The sample of white

DNA with 5*N*-HCl and 5% (w/v) TCA. The DNA was removed by centrifuging. One portion of the supernatant was used for the determination of total P (IV) and another for the determination of the ortho-P (V) by the modification of the method of Berenblum & Chain (1938) described by Ernster, Zetterström & Lindberg (1950). The precipitated DNA was extracted with 5% (w/v) TCA for 15 min. at 90°, as described by Schneider (1946). The total P was estimated in one portion of the extract (VI) and the DNA was estimated by ultraviolet absorption (VII) in another. Details

of this method are given in the Appendix. The residue remaining after the extraction of the DNA contained a negligibly small concentration of P.

The other pair of duplicate samples from which the acid-soluble P and lipid P had been removed was treated with 5% (w/v) TCA for 15 min. at 90°, as described by Schneider (1945). Portions of the TCA extract were used for the determination of DNA by the diphenylamine reaction of Dische (1930) (VIII), for the determination of PNA by the orcinol reaction of Mejbaum (1939) (IX), for the determination of total P (X), and for the determination of total nucleic acid by the method of ultraviolet absorption described in the Appendix (XI). A total P estimation was also done on the residue remaining after the TCA extraction (XII).

All fractions were made up in volumetric flasks, and portions containing 10–20 μ g. P were taken for each P determination. Total P was estimated by the method of King (1932). The colour was read in a Coleman Universal spectrophotometer at 650 m μ . The diphenylamine and

for the lipid P of white matter and grey matter of dog brain and cat sciatic nerve are of the same order as those reported for similar mammalian tissues (see Johnson, McNabb & Rossiter, 1948, 1949, for references). It will be noted that, as originally pointed out by Davidson & Waymouth (1944), the concentration of protein-bound P in white matter exceeds that in grey matter.

Table 4 compares the concentration of total nucleic acid determined by the Schmidt-Thannhauser method with that determined in the Schneider TCA extract by (a) the colour reactions, (b) the total P, and (c) the ultraviolet-absorption method. The values obtained by the Schmidt-Thannhauser method are all greater than those for the total P in the TCA extract and these, in turn, are greater than the values for the ultraviolet-absorption method. The greatest difference is in white

Table 3. *Distribution of phosphorus compounds in tissue from the nervous system*

(Mean $\pm$ s.e.m. of six determinations, expressed as mg. P/100 g. wet tissue.)

	Determination	Dog white matter	Dog grey matter	Cat sciatic nerve
Acid-soluble P	I	74.5 $\pm$ 4.2	79.5 $\pm$ 3.1	52.0 $\pm$ 2.1
Lipid P	II	333 $\pm$ 14	157 $\pm$ 6	308 $\pm$ 23
Protein-bound P	III	33.9 $\pm$ 2.4	24.6 $\pm$ 1.1	22.3 $\pm$ 1.0
Total P	I + II + III	441 $\pm$ 19	261 $\pm$ 10	382 $\pm$ 26

Table 4. *Total nucleic acid of tissue from nervous system*

(mg. P/100 g. wet tissue. Mean of six experiments $\pm$ s.e.m.)

	Determination	Dog white matter	Dog grey matter	Cat sciatic nerve	Measured
Schmidt & Thannhauser (1945)	III – V	31.3 $\pm$ 1.7	23.8 $\pm$ 0.6	20.1 $\pm$ 1.0	DNA + PNA + 'inositide P'
Schneider (1945), colour reactions	VIII + IX	21.1 $\pm$ 2.5	25.4 $\pm$ 1.2	14.9 $\pm$ 1.1	DNA + PNA + ?
Schneider (1945), phosphorus	X	18.8 $\pm$ 2.5	16.5 $\pm$ 0.3	14.0 $\pm$ 1.5	DNA + PNA + part of 'inositide P'
Ultraviolet absorption	XI	10.7 $\pm$ 0.5	15.2 $\pm$ 0.3	10.2 $\pm$ 0.5	DNA + PNA

orcinol reactions were performed as described by Schneider (1945), the final readings being made at 600 and 655 m μ , respectively. Results are all presented in terms of the P content of the standard solutions.

RESULTS

Comparison of methods

Table 3 shows the concentration of acid-soluble P, lipid P and protein-bound P in the white matter and grey matter of dog brain and the sciatic nerve of the cat. These values agree in most respects with those reported for the brain and nerve of the monkey by Bodian & Dziewiatkowski (1950), and for the sciatic nerve of the guinea pig by Samuels *et al.* (1951). The concentration of lipid P in white matter is more than twice that in grey matter. The figures

matter and the least in grey matter. The values given by the colour reactions are also considerably higher than those given by the method of ultraviolet absorption.

Tables 5 and 6 give the values obtained for the concentration of DNA and PNA by a number of different methods. For all three tissues there is good agreement between the figures for DNA obtained by the Schmidt-Thannhauser method, the modification of this method described by Schneider (1946), and the ultraviolet-absorption method. The only values that do not agree are those given by the diphenylamine colour reaction of Dische (1930). These are higher for all three tissues.

It was with the PNA that the greatest differences were found (Table 6). The values for the concentration of PNA obtained by the Schmidt-Thannhauser

method are all considerably greater than those obtained by the ultraviolet absorption method. The greatest difference is for white matter and the least is for grey matter. The values obtained by the orcinol colour reaction of Mejbbaum (1939) are also slightly greater for all three tissues ($P < 0.01$, in each instance). Table 6 also gives the values of two

extraction (XII), are greater than those found for the 'phosphoprotein' of Schmidt & Thannhauser (1945), i.e. the ortho-P liberated on hydrolysis in N-potassium hydroxide at 37° for 18 hr. (V). The difference is again greatest for white matter and least for grey matter, with the sciatic nerve being intermediate.

Table 5. *DNA of tissue from nervous system*(mg. P/100 g. wet tissue. Mean of six experiments $\pm$ S.E.M.)

	Determination	Dog white matter	Dog grey matter	Cat sciatic nerve	Measured
Schmidt & Thannhauser (1945)	III - IV	6.1 $\pm$ 0.2	4.5 $\pm$ 0.2	4.8 $\pm$ 0.2	DNA
Schmidt & Thannhauser (1945), modified by Schneider (1946)	VI	6.7 $\pm$ 0.2	4.7 $\pm$ 0.3	5.5 $\pm$ 0.2	DNA
Schneider (1945), colour reaction of Dische (1930)	VIII	14.2 $\pm$ 2.1	13.1 $\pm$ 1.5	6.6 $\pm$ 0.6	DNA + ?
Ultraviolet absorption	VII	5.8 $\pm$ 0.4	4.9 $\pm$ 0.4	5.4 $\pm$ 0.4	DNA

Table 6. *PNA of tissue from nervous system*(mg. P/100 g. wet tissue. Mean of six experiments $\pm$ S.E.M.)

	Determination	Dog white matter	Dog grey matter	Cat sciatic nerve	Measured
Schmidt & Thannhauser (1945)	IV - V	25.2 $\pm$ 1.6	19.3 $\pm$ 0.8	14.3 $\pm$ 0.8	PNA + 'inositide P'
Schneider (1945), colour reaction of Mejbbaum (1939)	IX	6.9 $\pm$ 0.4	12.4 $\pm$ 0.5	7.9 $\pm$ 0.7	PNA + ?
Combination I of Schmidt & Thannhauser (1945), and Schneider (1945)	IV - XII	10.7 $\pm$ 1.5	11.6 $\pm$ 0.7	8.1 $\pm$ 1.0	PNA + part of 'inositide P'
Combination II of Schmidt & Thannhauser (1945), and Schneider (1945)	X - VI	11.0 $\pm$ 2.3	11.1 $\pm$ 0.6	6.8 $\pm$ 0.5	PNA + part of 'inositide P'
Ultraviolet absorption	XI - VII	4.9 $\pm$ 0.2	10.3 $\pm$ 0.4	4.7 $\pm$ 0.5	PNA

Table 7. *'Phosphoprotein' of tissue from nervous system*(mg. P/100 g. wet tissue. Mean of six experiments $\pm$ S.E.M.)

	Determination	Dog white matter	Dog grey matter	Cat sciatic nerve	Measured
'Phosphoprotein' of Schmidt & Thannhauser (1945)	V	2.6 $\pm$ 0.2	2.4 $\pm$ 0.2	2.2 $\pm$ 0.2	PP ('phosphoprotein' phosphorus)
'Phosphoprotein' of Schneider (1945)	XII	15.3 $\pm$ 1.2	7.7 $\pm$ 0.6	10.2 $\pm$ 0.9	PP + part of 'inositide P'
'Inositide P'	III - (V + XI)	20.6 $\pm$ 1.7	8.3 $\pm$ 0.4	10.0 $\pm$ 1.0	'Inositide P'
'Inositide P'	(IV - V) - (XI - VII)	20.3	9.0	9.6	'Inositide P'

frequently used estimates of the concentration of PNA obtained by combining the procedures of Schmidt & Thannhauser (1945) and Schneider (1945). For white matter and peripheral nerve the figures are greater than those obtained with the ultraviolet absorption method, but for grey matter the differences are not statistically significant ($P = 0.2$ and 0.3 , respectively).

Table 7 shows that the values found for the concentration of 'phosphoprotein' of Schneider (1945), i.e. the phosphorus remaining after the TCA

If it is assumed that the ultraviolet-absorption values are the more reliable, it is clear that the method of Schmidt & Thannhauser (1945), while giving a good estimate of the concentration of DNA, gives a gross overestimate of the concentration of PNA, especially for white matter and, to a lesser extent, for peripheral nerve. Both the colour reactions and the phosphorus estimations of Schneider (1945) also give results that are too high. A hint as to the cause of some of these discrepancies is that the values for the concentration of 'phos-

phoprotein' of Schneider (1945) are much greater than those obtained by the method of Schmidt & Thannhauser (1945), especially for white matter (Table 7). It is clear that much of the phosphorus estimated as 'phosphoprotein' by the former method is measured as nucleic acid, probably as PNA, by the latter. It was decided, therefore, to investigate the extraction of nucleic acid by the Schneider procedure.

Extraction of phosphorus by 5 % (w/v) TCA at 90°

The acid-soluble P and lipid P were removed from four pairs of duplicate samples of cat sciatic nerve and the residue was treated for periods of 5, 10, 15 or 30 min., with 5 % (w/v) TCA at 90° as described by Schneider (1945). Fig. 1 shows that after 15 min.

Fig. 1. Effect of time of heating with 5 % (w/v) TCA at 90°, on the values obtained for the concentration of total nucleic acid in cat sciatic nerve by three different methods.
 ▲—▲—▲, colour reactions of Schneider (1945);
 ●—●—●, total P of extract (Schneider, 1945);
 ■—■—■, ultraviolet absorption method.

there was little increase in the concentration of nucleic acid in the extract, as measured by the colour reactions (VIII+IX) or ultraviolet absorption (XI), but that the total P in the extract (X) increased with time. At 15 min. (the usual time of extraction in the Schneider procedure) the values were relatively the same as those presented in Table 3. The figure for the colour reactions is the greatest, followed by that for the total P and the ultraviolet absorption, in that order. With an increase in the length of the extraction time, more

phosphorus was removed without any corresponding increase in the ultraviolet absorption, or the value given by the colour reactions. It is clear that some phosphorus compound not possessing the characteristic ultraviolet-absorption properties of nucleic acid or nucleotides was extracted by the TCA. There would thus appear to be no justification for the common practice of designating all phosphorus removed by extraction with TCA for 15 min. as nucleic acid P, and the phosphorus remaining in the residue after this time as 'phosphoprotein' P.

Fig. 2 gives the result of a similar experiment in which both the total P (X) and the ortho-P were measured in the extract and the total P (XII) was measured in the residue. Only a small fraction of the phosphorus of the extract was orthophosphate. In other experiments, in which the time of the extraction was prolonged (2 hr. or more), the residue P tended to zero and the ortho-P in the extract tended to the value found for the Schmidt-Thannhauser 'phosphoprotein' (V). After the usual extraction of 15 min. there was negligible ortho-P in the TCA extract.

Essentially similar results were obtained in a number of other experiments with cat sciatic nerve and with both white matter and grey matter of dog

Fig. 2. Effect of time of heating with 5 % (w/v) TCA at 90° on the total P and ortho-P of the extract, and the total P of the residue of cat sciatic nerve from which the acid-soluble P and lipid P had previously been removed.
 ●—●—●, total P of extract; ■—■—■, ortho-P of extract; ▲—▲—▲, total P of residue.

brain. With white matter more phosphorus was extracted by the TCA and the time necessary for complete extraction was longer. At 15 min. the value for the concentration of nucleic acid calculated from total P was often of the same order as that calculated from the colour reactions. With grey matter, on the other hand, the values given by the colour reactions were greater than those calculated from total P. In no instance were we able to confirm the report of Samuels *et al.* (1951) that the concentration of nucleic acid determined by ultra-

violet absorption agreed with the phosphorus values. With other tissues (e.g. pancreas and spleen) the agreement was good.

When the residue remaining after treatment with 5% (w/v) TCA at 90° for 15 min. was subjected to the Schmidt-Thannhauser hydrolysis almost all the material passed into solution and only a further small quantity, containing negligible phosphorus, was precipitated when the solution was neutralized and TCA added to make the final concentration 5%. Table 8 shows that very little of the phosphorus of the residue appeared in the hydrolysate as ortho-phosphate. The concentration of ortho-P was found to be similar to that obtained for the 'phosphoprotein' of Schmidt & Thannhauser (1945) (V) in a parallel experiment.

Table 8. *Residue phosphorus of cat brain after extraction with 5% (w/v) TCA for 15 min. at 90°*

	Experiment	
	1	2
Total residue P (XII)	8.2	11.3
After Schmidt & Thannhauser (1945) hydrolysis:		
Ortho-P	3.2	2.1
Organic P	5.0	9.2
'Phosphoprotein' of Schmidt & Thannhauser (1945) (V)	3.5	2.3

Similar concentrations of ortho-P are thus obtained when tissue from the nervous system, from which the acid-soluble and lipid P has been removed, is (a) hydrolysed by the method of Schmidt & Thannhauser (1945), (b) subjected to prolonged extraction (longer than 2 hr.) with 5% (w/v) TCA at 90°, and (c) treated with 5% (w/v) TCA at 90° for 15 min. and the residue subjected to the Schmidt-Thannhauser hydrolysis. The origin of this phosphorus is obscure. Presumably it is derived from an acid-insoluble compound which, like casein, yields ortho-P on hydrolysis. This phosphorus represents the 'phosphoprotein' of Schmidt & Thannhauser (1945) and will be referred to as PP.

The phosphorus, some of which remains in the residue after extraction for 15 min. with 5% (w/v) TCA at 90°, and which is slowly removed by further extraction, is undoubtedly determined as PNA in the Schmidt-Thannhauser procedure and presumably causes the high values when PNA is determined by this method (Table 6). The substance responsible for these high PNA values interferes in the same way as the protein-bound metaphosphate of yeast (Wiame, 1947, 1949; Schmidt, Hecht & Thannhauser, 1946). Protein-bound metaphosphate was not found in animal tissues by Ebel (1949) or Davidson, Frazer & Hutchison (1951).

However, a phosphorus compound with the requisite properties is the trypsin-resistant lipid-protein complex isolated from the white matter of calf brain by Folch & Le Baron (1951). This substance, which contains inositol diphosphate, is not removed from brain tissue by the usual ethanol-ether extraction, and Folch & Le Baron (1951) state that all of the phosphorus of this compound appears as PNA in the method of Schmidt & Thannhauser (1945). Folch (1951) states that this substance occurs chiefly in white matter of brain, and he believes it to be part of the classical 'neurokeratin' described by Kühne & Chittenden (1889) and thought to be a constituent of the myelin sheath of a mammalian nerve fibre. For further comments on the chemical nature of 'neurokeratin', see the paper by Folch & Lees (1951). The phosphorus of the protein-bound P (III) that cannot be accounted for either as nucleic acid or as PP will be referred to as 'inositide P'. It should be stressed that there is no evidence that all such phosphorus is present in brain as inositide, although it is shown (see below) that this type of phosphorus compound occurs in much greater concentration in white matter than in grey matter.

An estimate of the concentration of the 'inositide P' remaining after the removal of acid-soluble P and lipid P can be obtained by subtracting the sum of the values found for the PP (V) and the total nucleic acid determined by ultraviolet absorption (XI) from the protein-bound P (III). By subtracting the ultraviolet-absorption value for PNA (XI - VII) from the value obtained by the Schmidt-Thannhauser method (IV - V), another estimate of 'inositide P' can be obtained by a slightly different method.

Table 7 gives the concentration of 'inositide P' determined by each of these methods. By far the greatest concentration is in the white matter of dog brain, where 'inositide P' accounts for two-thirds of the protein-bound P. The concentration in grey matter and cat sciatic nerve is considerably lower.

*Extraction of chromogenic material by
5% (w/v) TCA at 90°*

Fig. 3 shows the results of an experiment similar to that represented in Fig. 1 in which the colour reactions of Dische (1930) and Mejbaum (1939) were carried out on TCA extracts of cat sciatic nerve. The extraction times were from 5 to 60 min. Although it is shown in Fig. 1 that the total nucleic acid determined by the colour reactions did not increase when the extraction time was increased from 15 to 30 min., Fig. 3 shows that after 15 min. the colour given by the diphenylamine reaction became less, and that given by the orcinol reaction became greater. These were consistent findings and were repeated in a number of similar experiments

with white matter and grey matter of dog brain as well as with cat sciatic nerve.

The lower values for the diphenylamine reaction with extraction times greater than 15 min. were obtained with tissues other than nerve or brain, although the higher values given by the orcinol reaction were peculiar to tissue from the nervous

Fig. 3. Effect of time of heating with 5% (w/v) TCA on the values obtained for the concentration of total nucleic acid, DNA and PNA in cat sciatic nerve by the colour reactions. $\blacktriangle$ — $\blacktriangle$ — $\blacktriangle$, total nucleic acid by addition; $\blacksquare$ — $\blacksquare$ — $\blacksquare$, DNA by method of Dische (1930); $\bullet$ — $\bullet$ — $\bullet$, PNA by the method of Mejbaum (1939).

system. With extracts of brain or nerve the colour produced in the diphenylamine reaction was a blue-green rather than the clear blue of the DNA standard. With white matter the peak of the absorption curve of the colour complex was the same as that of the standard, but the curve was much flatter. With grey matter the curve was greatly distorted and the peak was shifted to lower wavelengths. When the reaction was carried out on DNA isolated by the Schmidt-Thannhauser method, the

values obtained were similar to those given by total P (VI) or ultraviolet absorption (VII), and they were much lower than those obtained when the reaction was done on the Schneider TCA extract (VIII). The absorption characteristics of the colour complex given by the isolated DNA appeared normal. In the orcinol reaction the green colour obtained with brain extracts appeared the same as that given by the PNA standard, and the characteristics of the absorption curve of the colour complex were similar.

The results indicate that these two colour reactions are not suitable for estimating nucleic acid in TCA extracts of tissue from the nervous system.

DISCUSSION

It would thus appear that tissue from the nervous system contains at least two substances that interfere greatly with methods widely used for the determination of nucleic acids: (1) the 'inositide P' that is estimated as PNA by the method of Schmidt & Thannhauser (1945) and is measured, in part, as nucleic acid P by the method of Schneider (1945), and (2) chromogenic material that interferes with both the diphenylamine reaction of Dische (1930) and the orcinol reaction of Mejbaum (1939). Table 9 summarizes the substances that would be measured in each of the determinations outlined in 'Analytical methods'. In addition, some of the substances that would be measured in each of the estimates of total nucleic acid, DNA and PNA and 'phosphoprotein' given in Tables 4-7, can be found in the last column of the tables.

No attempt was made to determine whether a phosphorus compound similar to that which gives rise to the 'inositide P' occurs in tissues other than those of the nervous system. The experiments of Davidson *et al.* (1951) would suggest the possibility

Table 9. Substances estimated in each of the determinations outlined in 'Analytical methods'

Determination	Previously assumed	Estimated
I	Acid-soluble P	Acid-soluble P
II	Lipid P	Lipid P
III	DNA + PNA + 'phosphoprotein' of Schmidt & Thannhauser (1945)	DNA + PNA + PP + 'inositide P'
IV	PNA + 'phosphoprotein' of Schmidt & Thannhauser (1945)	PNA + PP + 'inositide P'
V	'Phosphoprotein' of Schmidt & Thannhauser (1945)	PP
VI	DNA of Schmidt & Thannhauser (1945)	DNA
VII	DNA by ultraviolet absorption	DNA
VIII	DNA by Dische (1930) diphenylamine reactions (Schneider, 1945)	DNA + ?
IX	PNA by Mejbaum (1939) orcinol reaction (Schneider, 1945)	PNA + ?
X	DNA + PNA by phosphorus (Schneider, 1945)	DNA + PNA + part of 'inositide'
XI	DNA + PNA by ultraviolet absorption	DNA + PNA
XII	'Phosphoprotein' of Schneider (1945)	PP + part of 'inositide P'

that smaller concentrations of such a compound may be present in liver. In most tissues the concentration of nucleic acid is considerably greater than in brain or nerve, and the Schneider 'phosphoprotein' (XII) represents a much smaller percentage of the total protein-bound P. The effect on the quantitative determination of nucleic acid would, therefore, be much less. However, Jeener (1949) and Davidson *et al.* (1951) have shown that after the administration of ^{32}P the specific activity of the Schmidt-Thannhauser PNA fraction of liver (our IV-V) is much greater than that of isolated PNA, even after the very active PP (our V) has been removed. These results suggest that liver contains small amounts of a metabolically active phosphorus compound that interfere with specific activity measurements made by the Schmidt-Thannhauser method. This interference is much greater in tissue of the nervous system. 'Inositide P' accounts for over 80% of the Schmidt-Thannhauser PNA (IV-V) in the white matter of dog brain (Table 5) and Strickland (1951), working in this laboratory, has shown that ^{32}P is incorporated into the PP and 'inositide P' of brain and nerve much more readily than into the DNA or PNA.

The gross differences between the estimates of the concentration of nucleic acids obtained by the methods of Schmidt & Thannhauser (1945), Schneider (1945) and by ultraviolet absorption are greatest in tissue of the nervous system. In a number of experiments with pancreas, spleen, thymus, white cells and reticulocytes, in which the same techniques and the same standards were used, fair agreement was obtained between the methods. This would strongly suggest that the differences observed for the nervous system are genuine. Since each of the three methods is dependent upon a different part of the nucleic acid molecule, it is at least theoretically possible that the lack of agreement is due to some fundamental difference in the structure of the nucleic acids of the nervous system. We are aware of no studies on the chemistry of nucleic acids isolated from the nervous system, but in view of recent studies on nucleic acid structure (i.e. Chargaff, 1951) such a possibility would appear to be unlikely.

Both Schneider (1946) and Tsuboi (1950) reported good agreement between various nucleic acid methods for a number of tissues, although it is apparent from the paper of Schneider (1946) that in rat brain the value for the concentration of total nucleic acid, determined by the Schmidt-Thannhauser method, is greater than that determined either by total P or by colour reactions done on the Schneider extract. The difference is due to an increase in the figure for PNA. Schneider (1945, 1946) also reported that for brain tissue the concentration of total nucleic acid in the TCA extract given by the

colour reactions agreed with the figure given by phosphorus estimations. This agreement is fortuitous. The phosphorus figures are too high because of the presence of some 'inositide P', and the figures given by the colour reactions are too high because of interfering chromogenic material.

The results obtained by the ultraviolet absorption method for the concentration of nucleic acid in brain and nerve are much lower than the figures published by previous workers (Kossel, 1882; Berenblum, Chain & Heatley, 1939; Rosenthal & Drabkin, 1943; Davidson & Waymouth, 1944; Schmidt & Thannhauser, 1945; Schneider, 1945, 1946; Schneider & Klug, 1946; von Euler & Hahn, 1948; Bodian & Dziewiatkowski, 1950; Samuels *et al.* 1951). In Table 10 our results are compared with those obtained in the recent studies of Bodian & Dziewiatkowski (1950) and Samuels *et al.* (1951). As already pointed out, our results for acid-soluble and lipid P in the white matter and grey matter of dog brain and cat sciatic nerve agree well with the published figures for monkey brain and guinea-pig nerve, but our figures for total nucleic acid, DNA, and 'phosphoprotein' are considerably lower.

It is of interest to note that the concentration of total nucleic acid in grey matter is considerably greater than that in white matter. White matter and grey matter of dog brain and cat sciatic nerve all contain similar concentrations of DNA, but there is a much higher concentration of PNA in grey matter. This is not surprising, since grey matter contains many more nerve cell bodies. These contain abundant Nissl material, which is believed to be rich in PNA. White matter and grey matter of dog brain and cat sciatic nerve also contain a similar concentration of PP (V).

The finding of Bodian & Dziewiatkowski (1950) that white matter contains more 'phosphoprotein' (our XII) than grey matter is confirmed, although the values for dog brain reported in Table 7 are somewhat lower, both for white matter and for grey matter, than the figures given by these workers for monkey brain. The high concentration of phosphorus in the 'phosphoprotein' fraction of white matter is due to the much greater concentration of 'inositide P', much of which remains in this fraction after the TCA extraction. The high concentration of 'inositide P' in white matter, taken in conjunction with our further finding that this substance disappears from a peripheral nerve during Wallerian degeneration, even although the concentration of nucleic acid increases (Logan, Mannell & Rossiter, 1952), gives added weight to the suggestion of Folch (1951) that the 'inositide P' is derived from the 'neurokeratin' of the myelin sheath. It is this substance and not nucleic acid that contributes to the high protein-bound P found in white matter by Davidson & Waymouth (1944).

Table 10. *Phosphorus compounds in tissue from nervous system. Comparison of present results with those in recent literature*

Observer	No. of animals	Tissue	(mg. P/100 g. wet tissue $\pm$ s.e.m.)					'Inositide P'	
			Acid-soluble P	Lipid P	Total nucleic acid	DNA	PNA		PP
(1) <i>White matter</i>									
Bodian & Dziewiatkowski (1950)	4 monkeys	Corpus callosum	50.5 $\pm$ 2.1	377 $\pm$ 12	18.8 (a)	12.4 $\pm$ 1.6 (b)	6.4 $\pm$ 2.2 (c)	23.2 $\pm$ 2.6 (d)	—
Present results	6 dogs	Chiefly corpus callosum	74.5 $\pm$ 4.2	333 $\pm$ 14	11.5 (e)	6.3 $\pm$ 0.4 (f)	5.3 $\pm$ 0.2 (g)	2.6 $\pm$ 0.2 (h)	19.8 $\pm$ 1.7 (i)
(2) <i>Grey matter</i>									
Bodian & Dziewiatkowski (1950)	4 monkeys	Caudate nucleus	86.0 $\pm$ 2.1	185 $\pm$ 12	21.5 (a)	9.6 $\pm$ 1.6 (b)	11.5 $\pm$ 2.2 (c)	12.1 $\pm$ 2.6 (d)	—
Present results	6 dogs	Grey cerebral cortex	79.5 $\pm$ 3.1	157 $\pm$ 6	16.4 (e)	5.3 $\pm$ 0.4 (f)	11.1 $\pm$ 0.4 (g)	2.4 $\pm$ 0.2 (h)	7.5 $\pm$ 0.4 (i)
(3) <i>Peripheral nerve</i>									
Bodian & Dziewiatkowski (1950)	5 monkeys	Brachial and sciatic nerves	34.5 $\pm$ 3.7	328 $\pm$ 14	—	—	—	—	—
Samuels <i>et al.</i> (1951)	26 guinea pigs	Sciatic nerve	50 $\pm$ 5	313 $\pm$ 45	19.4 $\pm$ 5 (j)	—	—	27.0 $\pm$ 5 (d)	—
Present results	6 cats	Sciatic nerve	52.0 $\pm$ 2.1	308 $\pm$ 23	11.0 $\pm$ 0.5 (e)	5.8 $\pm$ 0.4 (f)	5.1 $\pm$ 0.5 (g)	2.2 $\pm$ 0.2 (h)	9.2 $\pm$ 1.0 (i)

(a) By colour reactions (our VIII + IX).

(b) By Dische (1930) diphenylamine reaction (our VIII).

(c) By Mejbaum (1939) orcinol reaction (our IX).

(d) ‘Residue P’ after TCA extraction (our XII).

(e) By ultraviolet absorption (XI).

(f) By ultraviolet absorption (VII).

(g) By ultraviolet absorption (XI - VII).

(h) By Schmidt & Thannhauser (1945) (V).

(i) By calculation [III - (V + XI)].

(j) By P estimation according to Schneider (1945) (our X).

SUMMARY

1. The concentration of acid-soluble phosphorus, lipid phosphorus and protein-bound phosphorus was determined in the white matter and the grey matter of dog brain and in the sciatic nerve of the cat. In addition, the concentration of total nucleic acid, deoxypentosenucleic acid (DNA), pentosenucleic acid (PNA) and 'phosphoprotein' was determined by the methods of Schmidt & Thannhauser (1945), Schneider (1945), and by an ultraviolet absorption method.

2. The figures for total nucleic acid, DNA and PNA given by the method of Schneider (1945), and those for total nucleic acid and PNA given by the method of Schmidt & Thannhauser (1945), were greater than the corresponding figures given by the ultraviolet absorption method. With other tissues there was good agreement between the three methods.

3. An investigation of the causes of these discrepancies revealed that tissue of the nervous system contains (a) a phosphorus compound, probably the inositol-containing trypsin-resistant lipid-protein complex of Folch & Le Baron (1951), which is estimated as PNA by the method of Schmidt &

Thannhauser (1945) and is measured, in part, as nucleic acid phosphorus in the method of Schneider (1945), and (b) chromogenic material that interferes with the colour reactions in the method of Schneider (1945).

4. The values obtained for the concentration of nucleic acid in tissue from the nervous system by the method of ultraviolet absorption are considerably lower than those previously published in the literature. The concentration of DNA is of the same order in white matter and grey matter of dog brain and in cat sciatic nerve, but the concentration of PNA is much greater in the grey matter.

5. The concentration of 'phosphoprotein' (PP) is of the same order in white matter and grey matter of dog brain and in cat sciatic nerve, but the concentration of 'inositide P' is much greater in white matter.

6. Some comments on the determination of DNA and PNA in tissue from the nervous system by the method of ultraviolet absorption are given in the Appendix.

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