APPENDIX

A Note on the Determination of Deoxypentosenucleic Acid and Pentosenucleic Acid in Tissue from the Nervous System by Ultraviolet Absorption

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Schneider (1945) introduced the convenient method of extracting nucleic acids from tissue with 5% (w/v) TCA. Such an extract, which contains minimal quantities of protein, may be used for the determination of nucleic acid by ultraviolet absorption.

The tissue samples were prepared as described in 'Analytical methods'. The extinction of (a) the TCA extract containing the DNA (VII) and (b) the TCA extract containing the total nucleic acid (XI) was determined in a Beckman Model DU quartz spectrophotometer at 268.5 m μ . The concentration of nucleic acid (in mg. P/100 g. wet tissue) for both DNA and total nucleic acid was calculated on the assumption that both DNA and PNA have an ϵ (P) of 9850 at this wavelength (see below). The concentration of PNA was determined by difference.

The procedure presented certain difficulties worth recording.

(1) Change in absorption characteristics of standards on heating for 15 min. at 90° in 5% (w/v) TCA

After heating in 5% (w/v) TCA at 90° for 15 min. the absorption maximum of the standard DNA was shifted from 259 to 267 m μ . and the ϵ (P) at maximum absorption increased from 7300 to 9900 (Fig. 4). After neutralizing and buffering in 0.1 M-phosphate at pH 7 the absorption maximum returned to 263 m μ ., but the extinction remained high. The changes in the standard PNA were in the same direction, but much less marked. After neutralizing and buffering at pH 7 the absorption maximum returned to

Fig. 4. Ultraviolet absorption of standard DNA. —, in water; ---, after heating with 5% (w/v) TCA at 90° for 15 min.; - · - ·, after heating with TCA as above, neutralizing and buffering in 0.1 M-phosphate at pH 7.

Table 11. *Effect of heating standard preparations of DNA and PNA for 15 min. at 90° in 5% (w/v) TCA*

	Standards used		Calculated for data of Tsuboi (1950)	
	$\lambda_{\max.}$ (m μ .)	ϵ (P) at $\lambda_{\max.}$	$\lambda_{\max.}$ (m μ .)	ϵ (P) at $\lambda_{\max.}$
DNA:				
In distilled water	259	7300	259	6900
After heating for 15 min. at 90° in 5% (w/v) TCA	267	9900	—	—
As above, but neutralized and buffered in 0.1 M-phosphate at pH 7.0	263	9500	262	9600*
PNA:				
In distilled water	258	9300	259	8300
After heating for 15 min. at 90° in 5% (w/v) TCA	261	10700	—	—
As above, but neutralized and buffered in 0.1 M-phosphate at pH 7.0	259	11000	259	10000*

* Heated for 20 min. at 90° in 5% (w/v) TCA. Readings at 260 m μ .

259 m μ . (Table 11). Similar changes in the ϵ (P) and $\lambda_{\max}$ of the standard DNA and PNA solutions were observed after heating in 6% (w/v) HClO₄ at 90° for 15 min. Other workers have described changes in the ultraviolet absorption characteristics of nucleic acids after acid, alkaline, or enzymic hydrolysis (Warren & Chanutin, 1948; Tsuboi, 1950; Little & Butler, 1951).

Fig. 5. Ultraviolet absorption of standard DNA and standard PNA after heating with 5% (w/v) TCA at 90° for 15 min. — — —, DNA; —, PNA.

Tsuboi (1950) reported similar changes in the $\lambda_{\max}$ and ϵ of DNA and PNA solutions. Table 11 shows that the wavelength of maximum absorption, and the ϵ (P) of both the standard DNA and the standard PNA after treatment with TCA and neutralization, agree with results obtained by Tsuboi (1950).

The absorption curves of equal concentrations (on a P basis) of DNA and PNA were found to intersect at 268.5 m μ . when the measurements were made after heating in TCA without neutralization (Fig. 5). Accordingly, routine measurements were made at this wavelength where both the DNA and PNA have an ϵ (P) of 9850. This wavelength also has the added advantage that the absorption due to the TCA is much less than at 260 m μ . (see below).

(2) Ultraviolet absorption of TCA

Fig. 6 shows the great ultraviolet absorption of 5% (w/v) TCA. On the same graph is plotted the extinction of PNA (2 μ g. P/ml.). It can be seen that at 260 m μ . the extinction due to the TCA is of the same order as that due to the nucleic acid. As a result, all reference blanks and standards must contain exactly the same concentration of TCA as does the test solution. In addition, the method suffers from the disadvantage that heating at 90° for 15 min. causes con-

siderable destruction of TCA (Table 12), the TCA being broken down according to the equation

Because of this, the blanks and standards must be heated for exactly the same length of time as the test samples. Various attempts were made to obviate this difficulty. The TCA could be removed with solvents such as ether or ethyl acetate, but this was time-consuming and in samples of

Fig. 6. Ultraviolet absorption of TCA and standard PNA. — — —, 5% (w/v) TCA; —, PNA (2 μ g. P/ml.).

biological tissue emulsions proved troublesome. HClO₄ (6%, w/v), which is free from interfering ultraviolet absorption, was found to extract the nucleic acids. However, when HClO₄ was used with tissue from the nervous system, the extracts absorbed strongly in the region of 274–276 m μ , presumably due to protein or protein degradation products. Experiments with casein showed that, when the 5% (w/v) TCA was replaced by 6% (w/v) HClO₄, more than three times the amount of material absorbing at 274–276 m μ . was extracted.

Table 12. Extinction at 269 m μ . of 5% (w/v) TCA heated at 90°

Heating time (min.)	$E_{1\text{cm.}}^{5\%}$ at 269 m μ .	
	Exp. 1	Exp. 2
0	0.162	0.162
5	0.158	0.157
10	0.150	0.150
15	0.135	0.132
30	0.113	0.114
60	0.092	0.090

As a routine, therefore, no attempt was made to remove the TCA. At 268.5 m μ . the proportionate effect due to any slight difference between the concentration of TCA in the test solution and in the reference blank is much less than at 260 m μ . (Fig. 6).

(3) *Extraction of nucleic acids*

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

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

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

Chemical Studies of Peripheral Nerve During Wallerian Degeneration

3. NUCLEIC ACIDS AND OTHER PROTEIN-BOUND PHOSPHORUS COMPOUNDS

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

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

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

METHODS

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

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

RESULTS

Nerve section

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