when $20 \leq 1/d \leq 100$ and ϕ is the volume fraction of solute. For the concentration units used (g./100 ml. solution) this expression reduces approximately to

$$[\eta] 100 = 0.233 (1/d)^{1.698},$$

and from this the axial ratio ($1/d$) was found to be 77. This value represents a frictional ratio (f/f_0) of 3.5, assuming the particles to be prolate spheroids, and using the data of Herzog, Illig & Kudar (1933). (Any hydration effect has been ignored.) The molecular weight M was then calculated from the equation

$$M = 2.45 \times 10^{22} \bar{V}^{\frac{1}{3}} (f/f_0)^{\frac{1}{3}} [S/(1 - \bar{V}_p)]^{\frac{1}{3}}.$$

The frictional ratio (f/f_0) of 3.5 gave a value of about 90 000. If the molecule was assumed to be spherical and unsolvated (i.e. $f/f_0 = 1$), the value of 14 000 was obtained.

DISCUSSION

The results show that the polysaccharide is not obviously polydisperse and the high limiting-viscosity number, and the marked dependence of the sedimentation constant on concentration suggest that the molecules are very asymmetrical. (Factors causing a dependence of sedimentation constant on concentration have been discussed by Kinell & Rånby (1950), and Ogston & Woods (1953).) The value of 90 000 for the molecular weight calculated using the frictional ratio obtained from the viscosity measurements is regarded as being of the correct order of magnitude, whilst the figure of 14 000 obtained assuming the molecule is spherical represents the minimum value. The effect of hydration could, however, reduce the higher figure. Few measurements have been made on similar poly-

saccharides from other bacteria, but Record & Stacey (1948) have reported molecular weights of 171×10^3 , 540×10^3 , and 141×10^3 for pneumococcus types I, II and III polysaccharides. Results obtained with other polysaccharides have been reviewed by Greenwood (1952).

SUMMARY

1. The capsular polysaccharide from an amylo-lytic rumen streptococcus has been found to be homogeneous in the ultracentrifuge.

2. The material possesses a high limiting-viscosity number and hence a very extended shape in solution (the frictional ratio (f/f_0) = 3.5).

3. The molecular weight of 90 000 calculated using the frictional ratio obtained from the viscosity measurements is regarded as being more correct than that calculated assuming a spherical molecule.

The author wishes to thank Prof. E. L. Hirst, F.R.S., for his interest in this work, and the Rockefeller Foundation for a gift of apparatus.

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The Distribution and Metabolism of Phosphorus Compounds in Normal and Demyelinating Nervous Tissue of the Chicken

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(Received 4 November 1953)

Several studies have been reported on the composition and metabolism of the phospholipids and other phosphorus-containing compounds of the mammalian nervous system, both in health and during the classical Wallerian degeneration which nerve fibres undergo following transection (see Brante, 1949; and Johnson, McNabb & Rossiter, 1950).

Demyelinating changes also occur in man after poisoning with tri-*ortho*-cresyl phosphate (TOCP), and numerous extensive outbreaks of poisoning by

this compound have been described (Smith & Elvove, 1930*a, b*; Ter Braak, 1931; Germon, 1932; Sampson, 1938; Hunter, Perry & Evans, 1944). More recently, the biochemical effects (Earl & Thompson, 1952*a, b*; Earl, Thompson & Webster, 1953) accompanying the histological and clinical changes (Barnes & Denz, 1953) in experimental intoxication with this compound have been studied, and it has been found to be a potent inhibitor of the pseudo-cholinesterase present in nervous tissue.

Barnes & Denz (1953) have also shown in experimental animals that poisoning with other anticholinesterases, including di-isopropyl fluorophosphonate can cause very similar degenerative changes in the myelin of the spinal cord and nerve sheath, and paralysis occurring in man following intoxication by one of the 'organophosphorus' anticholinesterase insecticides has also been described by Bidstrup, Bonnell & Beckett (1953).

Since the studies of Brante (1949) and Johnson *et al.* (1950) have been confined to changes following nerve section, it seemed of interest to determine whether any change in phosphorus composition and metabolism of nervous tissue was brought about by these toxic agents. Furthermore, no report seems yet to have been made on the distribution and metabolism of phosphorus compounds in the nervous system of birds, although the hen has been used by several groups of workers as being the most suitable species in which to study, both *in vitro* and *in vivo*, the biochemical, histological and clinical effects produced by anti-cholinesterases in nervous tissue (Earl & Thompson, 1952*a, b*; Earl, *et al.* 1953; Barnes & Denz, 1953).

The classical use of both the pigeon and the hen for the study of thiamine deficiency, which also causes demyelination not unlike that following TOCP intoxication (Swank, 1940; Swank & Prados, 1942), provides yet another reason for obtaining biochemical data of this kind in an avian species.

A report is given here of the amount of phosphorus present in three fractions of spinal cord and sciatic nerve, namely, the acid-soluble fraction (acid-soluble P) the phospholipid fraction (lipid P) and the residue remaining after removal of acid-soluble compounds and phospholipids (residual P), and of the rate of incorporation of ^{32}P into these, *in vivo*, in normal chickens, and in chickens dosed with TOCP at varying intervals before administration of the isotope.

EXPERIMENTAL

Pairs of chickens of mixed breed, weighing between 1.5 and 3.5 kg. were used. Pure TOCP (Geigy Pharmaceuticals Ltd.), 1 ml./kg. body wt., was given to one of each pair by stomach tube, either 4 or 14 days before administration to both of ^{32}P * (100 μC as $\text{H}_3^{32}\text{PO}_4$ in 2 ml. 0.06M phosphate buffer, pH 7.3) by injection into the wing vein.

Lowering of the plasma cholinesterase level 1-3 days after dosage with TOCP, as compared with that immediately before dosing, was taken as an adequate index of absorption. Cholinesterase activity was measured manometrically (Ammon, 1933), using butyrylcholine perchlorate as substrate.

The hens were kept in separate cages on a stock diet until killed by decapitation. Blood was collected from the cut end of the carotid artery into a bottle containing heparin as anticoagulant. Spinal cord, between the atlas and mid-

thoracic region, was removed and the dura mater stripped off, leaving an almost blood-free preparation. In order to obtain a standard length, the main trunks of the sciatic nerves were dissected out between the knee joint and the vertebral foramina, and cleaned of connective tissue and fat. All tissues were weighed immediately after removal.

The technique for separation of the three fractions of phosphorus compounds was essentially that of Schneider (1945). A sample of spinal cord was dispersed in 11 times its weight of glass-distilled water in a $\frac{1}{2}$ in. test tube, into which was fitted a glass rod with a bulb on the end, of such size that only a very narrow clearance was left between it and the walls of the tube (Lloyd, personal communication). On moving the rod rapidly up and down, without allowing the bulb to break the liquid surface, a uniform dispersion was quickly produced. 10 ml. of this was transferred by bulb pipette to a 20 ml. test tube graduated at 15 ml., and to this was added 5 ml. of 30% (w/v) trichloroacetic acid (Hopkin and Williams, A.R.).

The paired, weighed nerves were finely minced with scissors and transferred to a $\frac{1}{2}$ in. tube with approximately 10 ml. 10% (w/v) trichloroacetic acid and the bulb-plunger used as for cord. The resulting suspension was quantitatively transferred to a test tube and made up to exactly 15 ml. with 10% trichloroacetic acid. To enable the separation of the three fractions of phosphorus compounds to be carried out without transfer of the residue after each stage, the test tubes were fitted with ground-glass joints so that a condenser or an extension tube could be attached as required (see below).

All tubes were closed with turn-over rubber caps and centrifuged. The supernatant liquid (15 ml.) contained the whole acid-soluble phosphorus fraction of the tissue taken. The residues were washed once with 10 ml. 10% trichloroacetic acid, and twice with 10 ml. water, being centrifuged each time and the washings discarded.

A condenser was fitted to the tube and lipid P extracted with three successive 5 ml. lots of a mixture of 3 vol. ethanol and 1 vol. ether, and each lot being refluxed for 5 min. in a boiling-water bath. The combined extracts, removed with a Pasteur pipette after centrifuging, were made up to 15 ml. with the solvent mixture.

The residue was digested in 1 ml. H_2SO_4 (Hopkin and Williams, MAR), and approximately 3 ml. of 100 vol. H_2O_2 (Hopkin and Williams, MAR) were added drop by drop until the contents of the tube became clear. The digest was made up to 15 ml. with water.

Acid-soluble phosphorus of plasma was analysed after precipitating proteins with a final concentration of 10% trichloroacetic acid.

Phosphorus analyses were carried out in duplicate on each fraction by a method based on that of Fiske & Subbarow (1925), on portions digested in 60% HClO_4 (w/v) (Hopkin and Williams, A.R.). Appropriate reagent blanks were prepared. Ascorbic acid (Hopkin and Williams, BP) in a final concentration of 0.1% was used as reducing agent, and the colours compared with standards.

Radioactivity counts were done on 10 ml. of each of the three fractions of nervous tissues and on the plasma extract, in a type M6 liquid β Counter, and recorded for a standard time on a conventional type of scaler and timing unit. Corrections were applied in each experiment for background counts and for radioactive decay occurring during the experimental period.

* Supplied by A.E.R.E., Harwell.

RESULTS

Table 1 gives the means of the phosphorus analyses on the three fractions obtained from spinal cord and sciatic nerves of normal chickens. Means of the corresponding values for the birds 4 and 14 days after poisoning did not differ significantly from these. Since the extraction procedure was carried out in one test tube without transfer, the only point at which phosphorus might have been lost was in the washing of the residue left after extraction of acid-soluble P, and as a check on this, determinations of total P were made on samples of the original spinal-cord dispersion. The mean result obtained was $517 \text{ (S.E. } \pm 6.43) \mu\text{g./100 mg.}$, which does not differ significantly from the calculated total given in Table 1. The mean of the differences between the calculated and determined totals for the spinal cord of each normal bird was only $7.4 \text{ (S.E. } \pm 6.9) \mu\text{g./100 mg.}$

The mean weight of the standard lengths of sciatic nerves for the birds 4 and 14 days after

poisoning did not differ significantly from that of the normal nerves.

Table 2 shows the rates of uptake of ^{32}P by the three fractions expressed as relative specific activities (RSA) $\times 10^3$, i.e.

$$\frac{\text{specific activity of acid-soluble P of tissue}}{\text{specific activity of acid-soluble P of plasma}} \times 10^3,$$

which gives an estimate of the rate of passage of ^{32}P across the blood-brain barrier, and

$$\frac{\text{specific activity of lipid P of tissue}}{\text{specific activity of acid-soluble P of tissue}} \times 10^3,$$

and

$$\frac{\text{specific activity of residual P of tissue}}{\text{specific activity of acid-soluble P of tissue}} \times 10^3$$

which give estimates of the rates of incorporation of the isotope into phospholipid and residual fractions, on the assumption that the phosphorus-containing precursor of the compounds in these fractions is a component of the acid-soluble fraction. Fig. 1 shows

Table 1. *Phosphorus distribution in chicken nervous tissue and blood plasma*

Mean $\pm$ S.E. of mean. Expressed as $\mu\text{g./100 mg.}$ wet wt. for tissue, and mg./100 ml. for plasma.

	Acid-soluble phosphorus	Phospholipid phosphorus	Residual phosphorus	Total phosphorus (by addition)	No. of birds
Plasma	4.0 ± 0.56	—	—	—	13
Cord	71.4 ± 3.45	369 ± 7.2	68.8 ± 1.88	509	13
Nerve	46.0 ± 2.98	254 ± 8.2	39.4 ± 2.91	339	10

Table 2. *Incorporation of ^{32}P into chicken nervous tissue*

Interval between TOCP dosage and ³² P injection (days)	No. of birds	Interval between ³² P injection and killing (hr.)	Mean 10 ³ RSA* ±s.e. of mean		
			Acid-soluble P	Phospholipid P	Residual P
Cords					
Normal control	4	4	30.9±6.6	21.7±3.6	181±17.5
4	2	4	26.2	17.3	148
14	2	4	23.7	21.2	189
Normal control	5	24	105±9.0	75.5±5.3	410±17.0
4	3	24	116.3	67.3	396
14	2	24	109.2	65.5	370.5
Normal control	4	72	256±24.5	125±12.1	549±73.7
4	2	72	223	112	504
14	2	72	263.5	103.7	374
Nerves					
Normal control	4	4	132±32.7	14.1±0.5	243±13.2
4	2	4	164	11.8	213
14	2	4	178	18.8	209
Normal control	4	24	382±80.6	45.7±2.5	307±31.0
4	2	24	500	44.9	326
14	2	24	486	52.6	334
Normal control	2	72	634	126	396
14	2	72	704	121.8	398

* Relative specific activity (RSA) for acid-soluble P of tissue = $\frac{\text{specific activity of acid-soluble P of tissue}}{\text{specific activity of acid-soluble P of plasma}}$, for phospholipid P = $\frac{\text{specific activity of lipid P of tissue}}{\text{specific activity of acid-soluble P of tissue}}$, and for residual P = $\frac{\text{specific activity of residual P of tissue}}{\text{specific activity of acid-soluble P of tissue}}$.

Fig. 1.

Fig. 2.

Figs. 1, 2. Incorporation of ^{32}P into phosphorus compounds of chicken nervous tissue. Acid-soluble P, $\blacktriangle$ — $\blacktriangle$; phospholipid P, $\bullet$ — $\bullet$ — $\bullet$; residual P, $\odot$ — $\odot$ — $\odot$. Ordinate = relative specific activity (RSA), i.e.

$$\text{for tissue acid-soluble P: } \frac{\text{specific activity of acid-soluble P of tissue}}{\text{specific activity of acid-soluble P of plasma}} \times 10^3,$$

$$\text{for tissue lipid P: } \frac{\text{specific activity of lipid P of tissue}}{\text{specific activity of acid-soluble P of tissue}} \times 10^3,$$

$$\text{and for tissue residual P: } \frac{\text{specific activity of residual P of tissue}}{\text{specific activity of acid-soluble P of tissue}} \times 10^3,$$

Abscissae = time in hr. between administration of ^{32}P and killing.

the time course of the incorporation of ^{32}P into the three fractions of spinal cord, and Fig. 2 the corresponding curve for nerve.

The incorporation of ^{32}P continues to increase, but at a declining rate, during the whole of the experimental period in all three fractions of both tissues. The RSA of the residual P increases faster initially than does that of lipid P (Figs. 1 and 2).

There is no evidence of inhibition of uptake in the poisoned birds in either cord or nerve. The RSA of the residual P of spinal cord from birds poisoned 14 days before administration of ^{32}P , and killed 72 hr. after, is some 30% below the corresponding control value; but in view of the large s.e. of the latter figure this difference cannot be regarded as significant.

DISCUSSION

Composition. No attempt has been made in this work to define exactly the constitution of the three fractions of phosphorus compounds extracted by Schneider's (1945) method. In particular the

residual fraction contains a variety of different types of compound. Logan, Mannell & Rossiter (1952a, b), working with mammalian white matter and peripheral nerve, found that the residual fraction contained a considerable amount of phosphorus in excess of that accounted for by nucleic acids and phosphoprotein. They suggested that this may be present as 'inositide phosphorus', which may be identical with the inositol-containing, trypsin-resistant lipoprotein complex isolated from white matter by Folch & Le Baron (1951).

The phosphorus contents of the fractions obtained from chicken nervous tissue differ from those reported for similar fractions obtained from mammalian species (Bodian & Dziewiatkowski, 1950; Samuels, Boyarsky, Gerard, Libet & Brust, 1951; Logan *et al.* 1952a; Logan, 1952). Whether there is also a qualitative difference in the composition of the fractions, when obtained from different species, cannot be stated without a more detailed analysis.

Metabolism. The intervals of 4 and 14 days between dosing with TOCP and administration of the isotope were chosen, because the only biochemical abnormalities so far detected in nervous tissue in intoxication with this compound are a profound inhibition of pseudo-cholinesterase, which is the predominant cholinesterase of white matter (Ord & Thompson, 1952; Thompson & Webster, 1954), and a lesser inhibition of tributyrinase (Earl *et al.* 1953). It has been shown by these authors that at 4 days after poisoning the pseudo-cholinesterase of brain and cord is maximally inhibited, while at 14 days paralysis and clearly demonstrable demyelination of the cord and nerve are present (Barnes & Denz, 1953).

Passage of ^{32}P from blood to tissues. The results suggest that this is slow in both cord and nerve since the relative specific activities of the acid-soluble P have not reached a maximum even after 72 hr. Bakay & Lindberg (1949), Sachs & Culbreth (1951), Rosenfeld & Beath (1952), also reported a slow passage of ^{32}P across the blood-brain barrier in mammals. From experiments showing a much faster incorporation of ^{32}P into the acid-soluble P of the central nervous system after intracisternal, as compared with intravenous, injection of the isotope, they consider that phosphate is made available to the central nervous system by a slow secretion into the cerebrospinal fluid.

Entry of ^{32}P from blood into chicken sciatic nerve is faster than into cord; the results obtained by Brierley (1952) in the rabbit were similar, the rates of entry into nerve and cord, respectively, being 11 and 1.3 times the rate of entry from blood to cerebral cortex.

Rate of incorporation of ^{32}P into phospholipid and residual phosphorus fractions. Many workers have reported that this is slow in brain as compared with other organs (Artom, Sarzana, Porrier, Santagelo & Segre, 1937; Changus, Chaikoff & Ruben, 1938; Chargaff, Olsen & Partington, 1940; Bodian & Dziewiatkowski, 1950; Johnson & Albert, 1953). Changus *et al.* (1938) found that fasted rats, given ^{32}P orally, showed a maximal increase in the amount of isotope present in brain phospholipid after 200 hr., while liver, kidney and intestine showed a high maximum incorporation in 10–12 hr. Fasting, for the duration of the experiment, did not appear to affect ^{32}P incorporation into brain phospholipid. The present findings, in the chicken, that the RSA of lipid P and residual P have not reached a maximum even at 72 hr. after injection of ^{32}P , appear to support the conclusion of these earlier workers, although no direct comparison with other organs was made.

Chargaff *et al.* (1940) and Dawson (1953) reported that the rates of ^{32}P incorporation differed widely between the constituents of the lipid fraction of

brain, and Strickland (1952) showed that phosphoprotein incorporated the isotope faster than the other constituents of the residual fraction of cat brain. It is possible, therefore, that phosphoprotein may contribute to the steep initial rise in RSA of the residual P from chicken cord and nerve seen in Figs. 1 and 2 and Table 2.

The rates of incorporation of ^{32}P into lipid P and residual P of chicken cord and nerve are in reasonable agreement with those reported by Samuels *et al.* (1951) for guinea pig, and their finding that entry into nerve phospholipid is initially slower than into cord phospholipid is confirmed for an avian species.

Effect of TOCP in ^{32}P incorporation. Other workers have studied the uptake of ^{32}P into the phosphorus compounds of peripheral nerve during Wallerian degeneration following nerve section in mammals (Bodian & Dziewiatkowski, 1950; Samuels *et al.* 1951). As far as is known, no investigations of this kind have been made in animals during thiamine deficiency, poisoning with organophosphorus compounds or other experimental procedures known to produce demyelinating lesions (see Cavanagh & Thompson, 1954). In the present work it is evident from Table 2 that TOCP in a dosage sufficient to produce severe paralysis in chickens in 14 days, accompanied by histologically demonstrable demyelination in spinal cord and peripheral nerves, and by 80–90% inhibition of pseudo-cholinesterase in these tissues, produces no significant change in ^{32}P uptake into the three fractions obtained from chicken spinal cord and sciatic nerve. Bodian & Dziewiatkowski (1950) found an increase in uptake of ^{32}P in nerve, degenerating as a result of section, in all three fractions, and suggested that this is related to the increase in Schwann cell population in this case. Nothing, however, is known as yet of the reaction of the Schwann cells in intoxication with TOCP. On the other hand, Sawyer (1946) has claimed that, following section of guinea pig sciatic nerve, the pseudo-cholinesterase of the peripheral stump is largely unaffected, and the work of Koelle (1951), Ord & Thompson (1952), and Thompson & Webster (1954) suggested the possibility that this enzyme is concerned with some aspect of myelin metabolism and that its inhibition by TOCP and certain other anti-cholinesterases may be causally related to the ensuing demyelination. The presence of an apparently normal uptake into the phospholipid and residual fractions of cord and nerve undergoing demyelination following TOCP administration does not, however, preclude the possibility that the metabolism of a component of a fraction has been interfered with, and this effect not detected by the technique used in these experiments. This would be conceivable, especially if the substance concerned

was of low metabolic activity. Taurog, Chaikoff & Perlman (1942) reported that incorporation of ^{32}P into the phospholipids of liver and kidney slices was inhibited by cyanide, carbon monoxide and anaerobiosis, while Schachner, Fries & Chaikoff (1942) found that hexose increased the incorporation into phospholipids of brain *in vitro*. Recently DeLuca, Rossiter & Strickland (1953) have shown that incorporation of the isotope into pentosenucleic acid of cat brain is dependent on an active phosphorylating mechanism. Incorporation is depressed by anaerobiosis and by inhibitors which suppress oxygen consumption, and is supported by pyruvate and lactate (Findlay, Rossiter & Strickland, 1953). The finding, therefore, of Earl *et al.* (1953), that TOCP used in the same dosage as in the experiments described in this paper had no effect on the pyruvate oxidase system of chicken spinal cord and brain, is consistent with the absence of effect on ^{32}P incorporation into lipid P and residual P.

SUMMARY

1. The phosphorus compounds in spinal cord and sciatic nerve of the chicken have been investigated, and the effect of a demyelinating anti-cholinesterase (tri-*ortho*-cresyl phosphate, TOCP) studied.

2. TOCP in dosage sufficient to cause paralysis, demonstrable demyelination and 80–90 % inhibition of pseudo-cholinesterase of cord and nerve in 14 days, does not affect the amount of phosphorus in the acid-soluble, phospholipid, and residual fractions.

3. The rates of incorporation of ^{32}P into the three fractions have been measured, and a comparison made with available data on mammals. The rates appear to be slow in both tissues, and although ^{32}P enters the acid-soluble phosphorus fraction of nerve 4–5 times as fast as spinal cord, the rates of incorporation into the phospholipid and residual fractions are similar in both tissues.

4. TOCP, in the dosage used, does not appear to affect the rates of incorporation into the three fractions. The significance of this finding is discussed.

I am grateful to Prof. R. H. S. Thompson for his constant help and encouragement and to Prof. G. Payling Wright for permission to use his apparatus for the radioactivity counts, and also for advice from him and Mr R. S. Morgan. My thanks are also due to Messrs Geigy Pharmaceuticals Ltd. (Manchester) for gifts of pure TOCP, the Ministry of Supply for a grant for the purchase of birds, the Medical Research Council for a grant for expenses, and to Messrs D. Jarvis and P. Hunt for valuable technical assistance.

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