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LOCALIZATION OF CHOLINESTERASES IN THE CHICKEN NERVOUS SYSTEM AND THE PROBLEM OF THE SELECTIVE NEUROTOXICITY OF ORGANOPHOSPHORUS COMPOUNDS

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Using the thiocholine method, a restricted survey has been made of cholinesterases in the spinal cord and brain stem of the chicken. No simple relation between sites of selective damage in organophosphorus neurotoxicity and centres of cholinesterase activity could be adduced. Moreover, no significant differences between species susceptible and insusceptible to poisoning by these compounds were found by this method. It is concluded that, while cholinesterase may well play an intermediary role in the intoxication, other factors determine the selective damage to certain neurones and their processes.

It is difficult at the present time to dissociate the anticholinesterase activity of certain organophosphorus compounds from being causally related to their peculiarly selective toxicity towards the nervous system of the chicken. It is, however, no longer possible to maintain the earlier view that the neuropathy and spinal tract degeneration are a direct consequence of selective pseudocholinesterase inactivation (Earl & Thompson, 1952a & b), the most important reason being the finding by Davison (1953a) that not all of those compounds which preferentially inhibit pseudocholinesterases produce delayed paralysis in chickens. On the other hand, it has been recently shown by Davies, Holland & Rumens (1960) that, so far as the di-alkyl substituted organophosphorus compounds are concerned, a relation exists between the possession by such a substance of a fluoride radicle and its ability to cause delayed paralysis. They postulated that cholinesterase might be playing an intermediary rôle in the process, combining with the inhibitor and thus releasing potentially toxic fluoride ions at critical sites.

Since it has been demonstrated that both in tri-*o*-cresyl phosphate (Cavanagh, 1954) and in diisopropyl phosphorofluoridate (dyflos) poisoning (Fenton, 1955) it is the nerve fibre which is primarily damaged and not as hitherto believed the myelin sheath, it would seem essential, if the hypothesis of Davies *et al.* (1960) is to carry any weight, to determine the topographical distribution of cholinesterases within the nervous system, paying particular regard to those centres which are damaged by the neurotoxic process. The necessity for this is particularly important

now that it has been demonstrated histochemically in the central nervous system of the rat (Koelle, 1954) that cholinesterase activity varies markedly from one centre to another. An essential prerequisite for the hypothesis mentioned above must be that an adequate quantity of cholinesterase of one type or another should be present in the areas known to be damaged. To fill this gap the present histochemical work was carried out. A brief survey of the distribution of cholinesterases in comparable areas in four laboratory mammals was also made to gain some idea as to whether the differing species sensitivity to organophosphorus compounds could have any basis in cholinesterase distribution.

MATERIALS AND METHODS

The histochemical method

Gömöri's (1952) variant of Koelle's (1950) technique with certain minor modifications was used.

Substrates. Acetylthiocholine iodide and butyrylthiocholine iodide (Light & Co.).

Selective enzyme inhibitors. Mipafox (N.N′-diisopropyl-diamidic-phosphorofluoridate); dyflos (diisopropyl-phosphorofluoridate); "62.C.47" (1:5-bis-(4 trimethyl (ammonium-phenyl) pentane-3-one diiodide); ethopropazine hydrochloride; Eserine (physostigmine). The concentrations at which these inhibitors were employed are detailed in the text and in Table 1.

Source and treatment of tissues. White Leghorn chickens 1 to 2 years old were killed by an intracardiac injection of pentobarbitone (Nembutal, Abbott). Brain stem and pieces of spinal cord with adherent dorsal root ganglia were placed in a corked pot and frozen rapidly to $-70°$ C in an acetone/carbon-dioxide-snow mixture. The sections were cut at 10 μ in a cryostat and flattened on to albuminized slides. They were dried for 1 hr at room temperature before incubation in the reaction fluids.

Final incubation solutions

The concentrations of copper sulphate, magnesium chloride, glycine and maleate buffer in the final reaction mixture were as in Gömöri's method. Satisfactory deposits were obtained when the sodium sulphate concentration was 30% (w/v) in the acetylthiocholine mixture and 25% (w/v) in the butyrylthiocholine mixture. 25 ml. of solution was used, and the *p*H was adjusted to 6.5 before the substrate, dissolved in 1 ml. of distilled water, was mixed in. The final substrate concentration was 2 mg/ml.

In the inhibitor studies the sections were incubated for 0.5 hr at 37° C in the buffered mixture without substrate but containing inhibitor. At the end of this period the substrate in 1 ml. of distilled water was mixed in.

The sections were incubated for 3 hr at 37° C, then rinsed briefly in saturated sodium sulphate before being immersed in dilute ammonium sulphide. Sections were carried through in pairs, one of which was counterstained with alum carmine, before they were dehydrated and mounted in Canada balsam.

RESULTS

Topographical distribution of cholinesterases in the chicken nervous system

Since our interest was primarily centred upon the problem of organophosphorus neurotoxicity, only those regions which contained susceptible nerve cells or their fibres were examined. These included all levels of the spinal cord, dorsal root ganglia and spinal roots, and brain-stem and cerebellum. The last were cut together in semi-serial section at 0.5 mm intervals up to the level of the optic lobes. The diencephalon, forebrain and peripheral nerves beyond the spinal roots were not examined in this study.

The following description is based upon incubation for three hours in acetylthiocholine or butyrylthiocholine. Such anatomical terminology as is used is taken from Papez (1929).

Acetylthiocholine as substrate

Spinal cord. Enzyme activity was localized in two chief sites:

(1) All nerve cells throughout the spinal cord without exception, whether in the grey columns (Fig. 1) or lying ectopically in the white matter, contained enzyme both diffusely in their cytoplasm and in small granular masses along their surfaces.

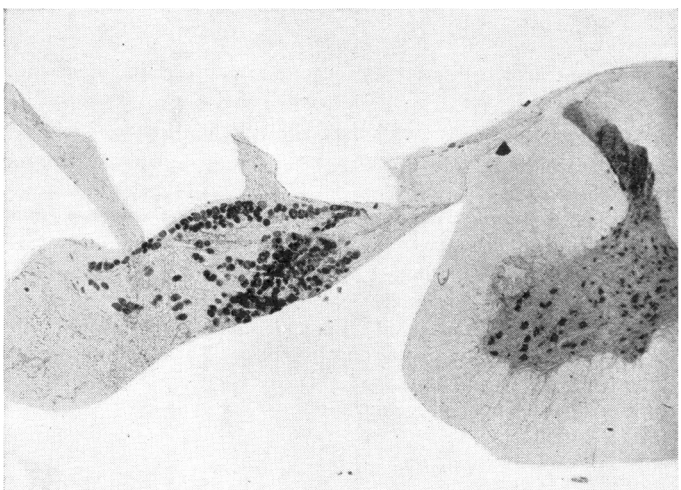

Fig. 1. Dorsal root ganglion and cervical spinal cord of chicken. Acetylthiocholine, ×21.

All cells appeared to contain an equal amount of deposit, although larger cells showed up more strikingly than smaller ones. The dorso-medial group (Clark's column) which gives rise to the spinocerebellar tracts was no different in its staining from other cell groups.

(2) The dorsal horns (Fig. 1) showed a diffuse localization of activity which was maximal in slender bands of fibres running into their medial aspect from the dorsal roots. This diffuse activity extended dorso-laterally into Lissauer's tracts and throughout the neuropil between the nerve cells. Such sites were again consistent at all levels in the spinal cord. No recognizable nerve cells were found in the dorsal caps of these horns, but small sparse cells in the intermediate zones stained exactly as nerve cells elsewhere. The fibres from the anterior grey columns extending into the white matter for short distances also stained, but no other fibre areas stained, with the exception of the fine fibres in immediate relation to ectopic subpial neurones.

Apart from these two major sites, the only other structures that stained were the walls of capillaries, but never vessels of larger calibre. No glial or ependymal cell was ever clearly stained in chicken tissues.

The nerve cells of the *dorsal root ganglia* all stained heavily (Fig. 1) and uniformly. Usually slight staining of their axons was visible.

Brain stem. Again staining occurred in two chief sites:

(1) All nerve cells stained without exception and with only minor variations in intensity (Fig. 2).

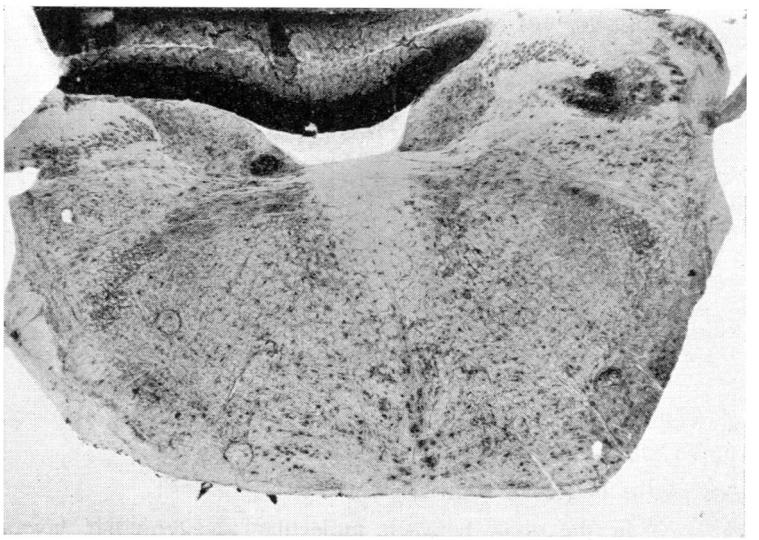

Fig. 2. Brain stem of chicken at level of lamellar nucleus. Acetylthiocholine, ×14.

(2) Well-demarcated fibre zones were stained in the following regions: (*a*) The expanded headward extension of the dorsal spinal horns in the lower medulla, and the descending root of the trigeminal nucleus ; (*b*) in the vestibular and acoustic nuclear regions both medially and laterally (Fig. 2) ; (*c*) a small unnamed zone in the ventral pons.

Except for some irregularly crossing fibres in the pontine reticular formation, all major pathways were unstained. A conspicuously unstained fibre region was the lamellar nucleus, in the centre of which was a band of stained nerve cells. This nucleus in silver impregnated preparations has a very dense felt-work of large fibres with large synaptic terminals terminating in the band of nerve cells. Its outflow, the dorsal cochlear commissure, was also unstained (Fig. 2).

Capillaries were conspicuously stained in all regions.

Cerebellum. The molecular layer throughout stained intensely and uniformly without any discernible structural pattern. Purkinje cells showed moderate activity (Fig. 6), while the cytoplasm of the cells of the granular layer also stained moderately intensely. There was no staining of white matter.

Butyrylthiocholine as substrate

A diffuse, unlocalized, faint staining occurred in most grey and white regions of *spinal cord* and *brain stem.* Sharp localization was found in the following areas.

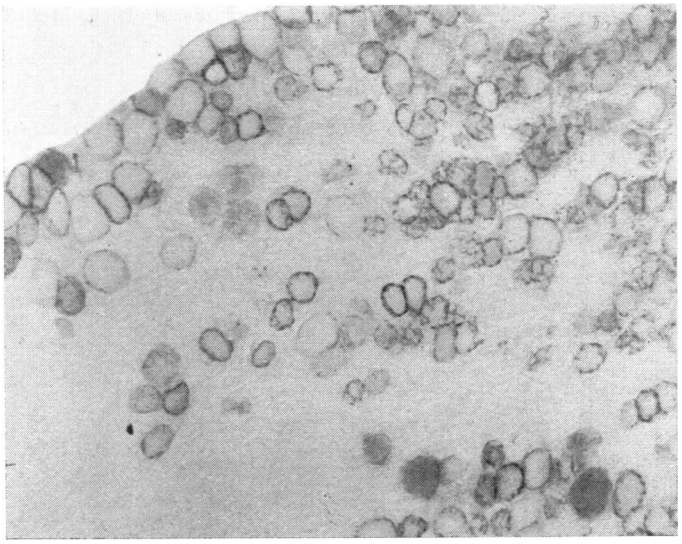

Fig. 3. Dorsal root ganglion of chicken. Butyrylthiocholine, × 82.

(*a*) Capsule cells of *dorsal root ganglia* (Fig. 3); slight staining also occurred in occasional nerve cells.

(*b*) *Choroid plexus* of fourth ventricle.

(*c*) Fibres lying in the zone between molecular and granular layers of the *cerebellum*, separating unstained Purkinje cells and sometimes running straight up into the molecular zone. These latter deposits from their position might either be part of the basket cell system around the Purkinje cell bodies, or more probably glial cells (astrocytes) which lie in this zone.

(*d*) Faint staining in the same regions that were stained when acetylthiocholine was used as substrate, that is, in fibre areas listed above and in all nerve cells. Prolonging incubation up to 6 hr caused moderate intensification of these last sites, but showed up no further structures. Evidence from the use of inhibitors and from poisoning birds with dyflos and tri-*o*-cresyl phosphate suggested that this staining was probably due to non-specific hydrolysis of butyrylthiocholine by true cholinesterase.

No staining occurred in any blood vessel, except capillaries, which, however, stained much less intensely than with acetylthiocholine and in this respect were comparable with nerve cells and fibre areas noted above. Glial cell bodies did not stain with either substrate, although it is possible that the diffuse staining seen with butyrylcholine may have been due to these cells.

Enzyme characterization by selective inhibitors

Incubation in acetylthiocholine alone provides the picture of total cholinesterase distribution because of the lack of specificity of this substrate (Holmstedt, 1957b). Furthermore, the action of eserine at 10^{-5} M concentration in abolishing all staining

with this substrate confirms that cholinesterases were responsible. Incubation in butyrylthiocholine alone, because of the specificity of this substrate towards pseudocholinesterase, should demonstrate the distribution of the latter enzyme, although since the specificity of butyrylcholine in the hen is less than it is for other species (Earl & Thompson, 1952a) doubts should be raised about this conclusion.

Prior incubation with dyflos at molar concentrations of 10^{-6}, 10^{-8} and 10^{-10} and using acetylthiocholine as substrate showed that staining in nerve cells and fibre areas was not significantly reduced at 10^{-8} M concentration, but it was abolished at 10^{-6} M concentration (Table 1). Moreover, using butyrylthiocholine all the diffuse

TABLE 1

EFFECT OF INHIBITORS ON HISTOCHEMICAL CHOLINESTERASE ACTIVITY

– No staining. + Staining significantly less than controls. ++ Staining identical with controls

		Acetylthiocholine		Butyrylthiocholine	
		Spinal cord and medulla		Capsule cells of spinal ganglia	Cells of cerebellar cortex
Inhibitor	Molar concentration	Nerve cells	Fibre areas		
Dyflos	1×10^{-6}	–	–	–	–
	1×10^{-8}	++	++	–	–
	1×10^{-10}	++	++	+	+
Mipafox	5×10^{-4}	–	–		
	5×10^{-6}	–	–		
	1×10^{-6}	+	+		
	5×10^{-7}	++	++		
	5×10^{-8}	++	++		
	1×10^{-8}	++	++		
"62.C.47"	1×10^{-3}	–	–		
	1×10^{-4}	+	+		
	2×10^{-5}	++	++		
	1×10^{-5}	++	++		
Ethopropazine hydrochloride	3×10^{-4}	+	+	–	–
	3×10^{-5}	++	++	–	–
	3×10^{-6}	++	++	++	++

staining noted with this substrate as well as the localized staining of capsule cells in spinal ganglia and the cells in the cerebellar cortex was lost at 10^{-8} M concentration of the inhibitor. Since it has been shown by Myers (1953) that pseudocholinesterase of birds is inhibited by dyflos at 10^{-8} M concentration, it is concluded that the staining from butyrylthiocholine was probably due to pseudocholinesterase activity. Conversely, most of the staining of nerve cells and fibre areas with acetylthiocholine was probably due to true cholinesterase activity, since staining was present until 10^{-6} M concentration of dyflos was used.

The action of varying concentrations of mipafox in the presence of acetylthiocholine (Table 1) confirmed that most of the staining with this substrate was probably due to true cholinesterase. Thus, no serious diminution of staining occurred at 5×10^{-7} M concentration, five times the concentration that Davison (1953b) found to block pseudocholinesterase activity in chicken brain. Staining was significantly reduced, however, at 10^{-6} M concentration and abolished at 5×10^{-6} M concentration.

The results with the reversible inhibitors " 62.C.47 " and ethopropazine, although somewhat less satisfactory, tended to support these conclusions. The latter substance in the presence of butyrylthiocholine completely abolished staining at 3×10^{-5} M concentration, but had little visible action at this concentration when acetylthiocholine was used. Bayliss & Todrick (1956) found that ethopropazine gave 99% inhibition of rat brain pseudocholinesterase at this concentration and none of the true cholinesterase, so that there may be little species difference in enzyme sensitivity to this inhibitor. The same, however, could not be said for the sensitivity to " 62.C.47." With acetylthiocholine as substrate we found that this compound only partially suppressed staining at 10^{-4} M concentration and completely suppressed it at 10^{-3} M concentration. By contrast, Bayliss & Todrick (1956) found with rat brain that true cholinesterase was inhibited 94% and 100% by 5×10^{-6} M and 2×10^{-5} M concentrations respectively. Species variation in the sensitivity of true cholinesterases to this group of substances seems to be appreciable, and it is noteworthy that Ferrari (1957) found bird cholinesterases much less sensitive than mammalian enzymes.

Results of poisoning birds with dyflos and tri-o-cresylphosphate

Two series of birds were poisoned with dyflos (0.8 mg/kg intramuscularly) and with tri-*o*-cresylphosphate (0.75 ml./kg orally). The birds treated with dyflos were protected from acute poisoning with oxime and atropine in the manner used by Davies *et al.* (1960). The effect on the histochemically demonstrable cholinesterase activity is shown in Table 2.

TABLE 2

RESPONSE TO POISONING WITH NEUROTOXIC ORGANOPHOSPHORUS AGENTS

— Negative. + Staining less than controls. ++ Staining identical to controls

		Acetylthiocholine		Butyrylthiocholine	
		Spinal cord and medulla		Capsule cells of spinal ganglia	Cells of cerebellar cortex
		Nerve cells	Fibre areas		
Dyflos 0·8 mg/kg i.m.	24 hr	++	++	—	+
	7 days	++	++	—	+
	12 days (paralysed)	++	++	++	++
Tri-*o*-cresyl phosphate 0·75 ml./kg orally	24 hr	++	++	—	—
	7 days	++	++	—	+
	12 days (paralysed)	++	++	—	+
	16 days (paralysed)	++	++	++	++

With acetylthiocholine as substrate no diminution of activity in any area at any time after poisoning was found. With butyrylthiocholine all evidence of activity disappeared from the capsule cells of the dorsal root ganglia, to return at the 12th day after dyflos and the 16th day after tri-*o*-cresylphosphate poisoning. The general diffuse staining also disappeared and returned in parallel with this localized activity. Paralysis was already present in the birds at these times. The response of the cerebellar staining with butyrylthiocholine was anomalous. One day and 7 days after dyflos there appeared to be a moderate reduction in intensity of staining,

while after tri-*o*-cresylphosphate there was no staining at 1 day and moderate staining from the 7th day onwards. Furthermore, the slight staining of nerve cells and fibre areas with butyrylthiocholine as substrate was not inhibited.

Comparison with various mammalian nervous systems

In view of the virtual restriction of the chronic neurotoxic effects of organophosphorus compounds to the chicken, cat and man, it was of interest to see whether the cholinesterase distribution was different in the chicken and cat from that of insusceptible species. The description by Koelle (1954) of the cholinesterase distribution in the rat nervous system suggested that this might be so. To this end the cervical enlargement of the spinal cord with attached dorsal root ganglia and the medulla were examined from the rat, the guinea-pig, the rabbit and the cat. The tissues were prepared exactly as were those of the chicken. The following differences were encountered in acetylthiocholine-incubated tissues, and these have been briefly referred to elsewhere by Cavanagh & Holland (1961).

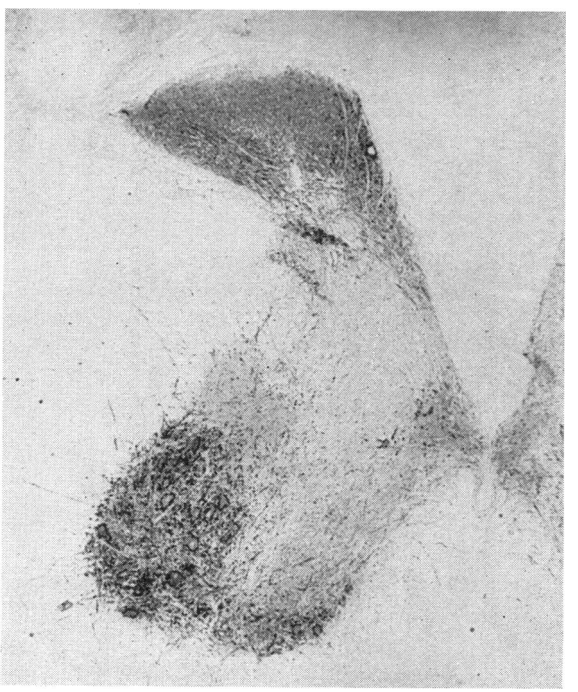

Fig. 4. Guinea-pig cervical spinal cord. Acetylthiocholine, ×52.

Spinal cord. Whereas in the chicken all nerve cell bodies without exception showed cholinesterase activity, this was not true for the mammals. Enzyme activity was shown to be concentrated *around* the neurones of the ventrolateral region of the grey columns, and only slight activity was recognizable in the neuronal cytoplasm (Fig. 4). The neurones in the dorsal and medial regions of the grey column contained no cholinesterase activity or had none in their immediate vicinity. By contrast

there was always a heavy concentration of activity in the synaptic regions of the posterior horns in both the mammals and the chicken (Fig. 4). Nerve fibres emerging from the ventrolateral cell group usually had conspicuous cholinesterase activity, and especially in the rat, rabbit and guinea-pig the fibres of the ventral roots were also stained. Dorsal root fibres were never stained, although nerve cells in the ganglia showed variable cholinesterase activity. In the rat a narrow band of fibres near the midline of the posterior columns appeared to contain cholinesterase.

Brain stem. Again, in contrast to the findings in the chicken, not all the mammalian nerve cells contained cholinesterase either in their cytoplasm or in their immediate vicinity. As Koelle (1954) found in the rat, many centres are either completely devoid of or show relatively little activity. On the other hand, certain regions, considered to be synaptic areas, that were well stained in the chicken, also showed heavy staining in the mammals (Fig. 5).

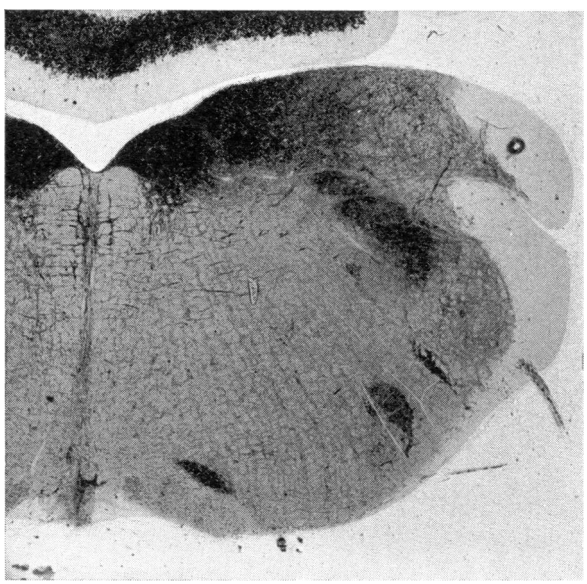

Fig. 5. Brain stem and cerebellum of rabbit. 2 hr in acetylthiocholine. Deposits in synaptic zones of vestibular areas and around cells of cranial nerve nuclei. ×12.5.

Cerebellum. The most striking differences were met with in the cerebellar cortex. The molecular (synaptic) layer was strongly positive in chicken and guinea-pig, but negative in rat, rabbit and cat. The Purkinje cells were positive in the chicken (Fig. 6) but negative in the other species. The granule cells, which stain poorly and somewhat diffusely in the chicken, were densely stained in rabbit (Fig. 6) and cat, less densely in guinea-pig and rat.

It is clear from sampling these two regions that differences of the greatest magnitude appear to exist in the cholinesterase distribution between chicken and mammals. On the other hand, certain basic similarities occur, particularly in regions of

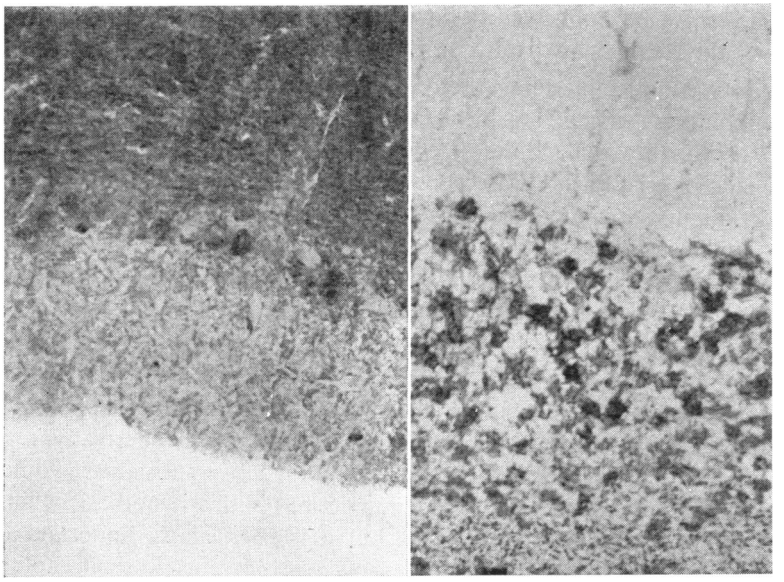

Fig. 6. Cerebellar cortex. Chicken left; rabbit right. Molecular layer at top; granular layer middle; white matter at bottom. Acetylthiocholine, ×130.

importance to the probable mechanism of organophosphorus neurotoxicity, such as the posterior grey horns of the spinal cord, which render such differences of less importance to this problem. This is also underlined by the findings in the cat, another species susceptible to organophosphorus intoxication, which does not appear greatly to differ from other mammals in its cholinesterase distribution.

DISCUSSION

The present experiments were done in order to determine whether or not a simple relation existed between distribution of cholinesterases, either true or pseudo, and the sites of damage produced by single doses of certain organophosphorus compounds. That such a relation does not exist cannot necessarily be inferred from the data presented here, although it might be fairly stated that the likelihood of its existence is not strengthened thereby. The histochemical method both for the detection of enzyme activity and for enzyme identification is fraught with uncertainties. The use of selective inhibitors, as Holmstedt (1957a) has pointed out, under conditions quite different from those used for studying enzyme characteristics by manometric methods, diminishes their value as a means of separating and identifying enzymes unless these are checked by parallel *in vitro* studies. None the less, so far as true cholinesterase is concerned, it would appear from the data given above that this enzyme is probably recognizable in all nerve cell bodies of chicken spinal cord, brain stem and cerebellum in a uniform manner, and the great variations in activity from one nuclear group to another met with by Koelle (1954) in the rat do not appear to exist in the chicken. This finding itself is of some interest to the problem of organophosphorus neurotoxicity, for should

true cholinesterase be implicated, as it may well be with phosphorofluoridates, all neurones would thus be equally at risk to their neurotoxic effects.

So far as pseudocholinesterase activity is concerned, the results obtained here were disappointing; except for localization in the capsule cells of spinal ganglia and in cells, probably glial, in the cerebellar cortex, no discrete activity was found. A similar lack of localization was found by Pepler & Pearse (1957) in the rat other than in certain hypothalamic neurones. This may in part be due to the levels of pseudocholinesterase activity, which are certainly much lower than those of true cholinesterase in the chicken nervous system (Earl & Thompson, 1952b).

The hypothesis put forward by Davies *et al.* (1960) to explain the role of cholinesterase in the neurotoxicity implies that the enzyme with which the organophosphorus compound combines should be present in the region where the subsequent biochemical lesion is produced by the liberated "toxophore" group, that is, fluoride with the phosphorofluoridates and cresolic derivatives with the aryl phosphates, for it is unlikely that such highly reactive substances would diffuse far before producing their effects. It is immaterial for this hypothesis whether true or pseudocholinesterase acts in this role, but it is particularly important to know whether the selectively damaged neurones show any difference in their cholinesterase content from those that appear to be immune.

While the question as to whether pseudocholinesterase is also present or not in neurones and other centres liable to damage is unimportant where the phosphorofluoridates are concerned, it is of some concern when the aryl phosphates are considered, for Earl & Thompson (1952a & b) have been unable to demonstrate depression of true cholinesterase activity in the chicken either *in vitro* or *in vivo* with tri-*o*-cresyl phosphate. Whether this is an absolute preference for pseudocholinesterase or whether, as with the phosphorofluoridates, the preference is relative is an important point worth re-examination. The finding of Carpenter, Jenden, Shulman & Turman (1959) that in rabbits chronically intoxicated with aryl phosphates there is a steady decline of total blood cholinesterase would suggest that the preference may be a relative one, and factors such as dose, route of administration and absorption must be taken into account. Until this point is determined it is not easy to put the aryl phosphates into line with the phosphorofluoridates.

Whether any pseudocholinesterase coexists with true cholinesterase in nerve cells or synaptic centres in the central nervous system of the chicken is difficult to determine histochemically. Holmstedt (1957b) has shown that such coexistence occurs in certain autonomic neurones of the cat, and Pepler & Pearse (1957) have demonstrated histochemically that certain neurones of the hypothalamus of the rat contain pseudocholinesterase. Where pseudocholinesterase, as demonstrated by substrate specificity and selective inhibitor concentrations, exists alone and at relatively high levels of activity such as in the capsule cells of the spinal ganglia, there is little difficulty in identifying it. Where the levels of activity are relatively low the insensitivity of the method limits its usefulness, and this probably accounts for the failure to reveal clearly pseudocholinesterase in those regions such as white matter where it is known to exist. Yet the method may not wholly be at fault, and factors such as distribution of the enzyme within the cell, species variation in

activity towards the substrate and the adequacy of the incubation medium must be taken into account. So far as our findings with each substrate and with inhibitors go, however, no evidence could be found that any of the staining in nerve cell bodies or in the synaptic areas around nerve cells or in posterior horns of spinal cord was the consequence of pseudocholinesterase activity. This does not, of course, completely exclude the possibility of this enzyme being present in small quantities or in a form not disclosed by the method. These qualifications are necessary for any negative result given by histochemical methods.

The sensitivity of the chicken, cat and man to organophosphorous neurotoxicity and the insusceptibility of rodents is one of the more intriguing aspects of this problem. Neither rat nor guinea-pig show paralysis, and in the rat the activity of pseudocholinesterase may be depressed for many weeks without serious consequences (Mendel & Myers, 1952). The anatomical distribution of damaged fibres after tri-*o*-cresyl phosphate poisoning in the chicken and in man would appear to be closely analogous (Cavanagh, 1954 ; Aring, 1942), and it is probable that the damage in the nervous system of the cat may be similar, although there is less precise information about this species (Smith & Lillie, 1931 ; Kidd & Langworthy, 1933). If, as the hypothesis of Davies *et al.* (1960) implies, intoxication by phosphorofluoridates of the neurone or its fibre endings is the simple result of release of fluorine ions interfering with a normal metabolic process, lack of correlation between susceptibility to paralysis and any particular species distribution of cholinesterase would suggest that species variation in response to poisoning may reflect a differing sensitivity of the neurones to the postulated toxic radicle rather than to any other factor. Moreover, distribution of cholinesterases does not offer any explanation for the finding that in the chicken as in man it is the distal portions of the long spinocerebellar and ventral descending tracts of the spinal cord of the chicken that are affected. If mere proximity to centres of high cholinesterase content were important a totally different distribution of lesions would be expected. It would therefore appear that, while cholinesterases may be essential to the toxic process, the distribution of structural damage is likely to be controlled by other factors.

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REFERENCES

ARING, C. D. (1942). Systemic nervous affinity of triorthocresylphosphate (Jamaica Ginger paralysis). *Brain*, **65**, 35–47.

BAYLISS, B. J. & TODRICK, A. (1956). The use of selective acetylcholinesterase inhibitor in the estimation of pseudocholinesterase activity in rat brain. *Biochem. J.*, **62**, 62–67.

CARPENTER, H. M., JENDEN, D. J., SHULMAN, R. & TURMAN, J. R. (1959). Toxicology of a triarylphosphate oil. *Arch. Ind. Health*, **20**, 62–80.

CAVANAGH, J. B. (1954). The toxic effects of triorthocresylphosphate on the nervous system. An experimental study in hens. *J. Neurol. Neurosurg. Psychiat.*, **17**, 163–172.

CAVANAGH, J. B. & HOLLAND, P. (1961). Cholinesterase in the chicken nervous system. *Nature (Lond.)*, in the press.

DAVIES, D. R., HOLLAND, P. & RUMENS, M. J. (1960). The relationship between the chemical structure and neurotoxicity of dialkyl organophosphorus compounds. *Brit. J. Pharmacol.*, **15**, 271–278.

DAVISON, A. N. (1953a). Inhibition of the cholinesterase of the central nervous system by organophosphorus compounds. *Biochem. J.*, **54**, p. xix–xx.

DAVISON, A. N. (1953b). Some observations on the cholinesterases of the central nervous system after the administration of organophosphorus compounds. *Brit. J. Pharmacol.*, **8**, 212–216.

EARL, C. J. & THOMPSON, R. H. S. (1952a). Triorthocresylphosphate and cholinesterases. *Brit. J. Pharmacol.*, **7**, 261–269.

EARL, C. J. & THOMPSON, R. H. S. (1952b). Cholinesterase levels in the nervous system in triorthocresylphosphate poisoning. *Brit. J. Pharmacol.*, **7**, 685–694.

FENTON, J. C. B. (1955). The nature of the paralysis in chickens following organophosphorus poisoning. *J. Path. Bact.*, **69**, 181–189.

FERRARI, W. (1957). Insensitivity of chicken cholinesterase to specific inhibitors of true and pseudo-enzyme. *Nature (Lond.)*, **180**, 144–145.

GÖMÖRI, G. (1952). *Microscopic Histochemistry*. University of Chicago Press.

HOLMSTEDT, B. (1957a). A modification of the thiocholine method for the determination of cholinesterase. I, Biochemical evaluation of selective inhibitors. *Acta physiol. scand.*, **40**, 322–330.

HOLMSTEDT, B. (1957b). A modification of the thiocholine method for the determination of cholinesterase. II, Histochemical application. *Acta physiol. scand.*, **40**, 331–337.

KIDD, J. G. & LANGWORTHY, O. R. (1933). Jake paralysis. *Bull. Johns Hopkins Hosp.*, **52**, 39–60.

KOELLE, G. B. (1950). The histochemical differentiation of types of cholinesterase and their localisation in tissues of the cat. *J. Pharmacol. exp. Ther.*, **100**, 158–179.

KOELLE, G. B. (1954). The histochemical localisation of cholinesterases in the central nervous system of the rat. *J. comp. Neurol.*, **100**, 211–236.

MENDEL, B. & MYERS, D. K. (1952). Pseudocholinesterase of brain. *Nature (Lond.)*, **170**, 928–929.

MYERS, D. K. (1953). Studies on cholinesterase. *Biochem. J.*, **55**, 67–78.

PAPEZ, J. W. (1929). *Comparative Neurology*. New York: T. Y. Crowell Company.

PEPLER, W. J. & PEARSE, A. G. E. (1957). The histochemistry of the esterases of rat brain, with special reference to those of the hypothalamic nuclei. *J. Neurochem.*, **1**, 193–202.

SMITH, M. I. & LILLIE, R. D. (1931). Histopathology of triorthocresylphosphate poisoning. *Arch. Neurol. Psychiat. Chicago*, **26**, 976–992.