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EFFECT OF OXIMES AND ATROPINE UPON THE DEVELOPMENT OF DELAYED NEUROTOXIC SIGNS IN CHICKENS FOLLOWING POISONING BY DFP AND SARIN

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Abstract—The oximes, 2-hydroxyiminomethyl-*N*-methylpyridinium methyl methanesulphonate (P2S) and the dodecyl iodide salt (PAD) and atropine sulphate have been shown to be without effect upon the development of delayed neurotoxic signs in chickens after poisoning by diisopropyl phosphorofluoridate (DFP). The production of delayed neurotoxic signs by repeated sub-neurotoxic doses of DFP and isopropyl methylphosphonofluoridate (Sarin) and the effect of varying the time interval between these doses have been demonstrated in chickens. Sub-neurotoxic doses of DFP and Sarin appear to be additive in their action and the interval between successive intoxications may be quite long—16 days with DFP.

AN EFFECTIVE antidote to the lethal effects of some anticholinesterase organo–phosphorus (O–P) compounds is a combination of the oxime 2-hydroxyiminomethyl-*N*-methylpyridinium methyl methanesulphonate (Pralidoxime Mesylate, P2S) and Atropine.[1] Many of these anticholinesterases are also neurotoxic and, in some, the dose necessary to produce delayed neurotoxicity is greater than that necessary to produce lethality. Thus, protecting against the lethal effects may result in tolerance to a large dose which may, in itself, produce delayed neurotoxic effects. Although it is the general impression that the use of P2S does not in any way influence the development of neurotoxic signs, the precise effect of this has not been fully examined. Thus Henschler[2] has claimed that one particular oxime, 2-hydroxyiminomethyl-*N*-methyl pyridinium dodecyliodide (PAD) has an antidotal action upon the paralysis produced by tri-ortho-cresyl phosphate (TOCP).

It has also been demonstrated that the repeated administration of sub-neurotoxic doses of various (O–P) compounds, which are highly lethal, may result in ataxia and paralysis.[3, 4] However, none of these investigations were designed to examine the quantitative problems involved. Smith and Elvove[5] who carried out a limited series of observations with TOCP showed that doses of only 1/40 or 1/50 that of a single dose necessary to produce neurotoxic effects would, if continued long enough, produce incapacitation.

The following studies have therefore been carried out to investigate; (a) the influence of P2S plus atropine and P2S plus PAD plus atropine upon the development of delayed neurotoxicity produced by DFP in chickens; (b) the cumulative effects of giving subneurotoxic doses of DFP and Sarin to chickens.

MATERIALS AND METHOD

Diisopropyl phosphorofluoridate (DFP), isopropyl methylphosphonofluoridate (Sarin), P2S and PAD were prepared at the Chemical Defence Establishment, and each was more than 95 per cent pure. Atropine sulphate powder was obtained from BDH Chemicals. Before use Sarin and DFP solutions were formulated in aqueous solution such that 0·1 ml contained 1·0 mg of each substance. P2S and atropine sulphate were also formulated in aqueous solutions such that a 1·0 ml aliquot of a combined solution contained 1·0 mg atropine sulphate and 100 mg P2S. PAD was formulated in iso-propanol solution to give a concentration of 100 mg/ml. Chickens of both sexes and aged between 12 and 18 months were used.

All injections of DFP, Sarin, P2S, PAD and atropine were intramuscular (i.m.) into the breast muscle of the chicken. For protection of the chickens against acute lethal effects, P2S and PAD (100 mg/kg) and atropine (1·0 mg/kg) were administered 10 min prior to DFP and Sarin which were given in various doses as described in the text. Any deviation from this procedure is indicated in the appropriate section.

Three criteria of neurotoxicity were adopted to assess the effect of a given procedure; (a) the time to onset; (b) the incidence; and (c) the severity of neurotoxic signs in individual birds.

Birds were kept in individual cages large enough to permit free movement. They were inspected, each day after dosing, by two independant observers; from the 7th day after dosing the procedure was standardized as follows. The birds were transferred to a large paddock and allowed complete freedom for several hours before detailed examination of individual birds was made. This consisted of making each bird walk along a narrow earthed passage for several min. The character of the gait was then separately assessed by two observers on the following scale: slight but definite clumsiness with incoordination 2 points; ataxia described as a drunken gait without serious incapacitation 4 points; marked ataxia with a waddling gait and inability to maintain an upright stance together with the bird frequently shuffling on its hocks 6 points; total inability to rise or walk scored 8 points.

A bird was first said to be positively affected when signs of incoordination and ataxia were obvious to each observer and the day when this occurred was taken to measure the latent period between dosing and onset of neurotoxic signs.

The assessment of disability for each individual bird on each day was the mean of each observers independant score. The final assessment of severity was the mean of the markings on 3 consecutive days when, in the opinion of the observers, the condition was steady and non-progressive. For each group of birds the mean severity of the condition on each day was assessed by dividing the total number of points recorded by the number of birds in the group.

RESULTS

The effect of P2S, PAD and atropine alone in chickens. Neither P2S nor atropine, in the doses used, produced any abnormal signs in the non-poisoned bird.

Four birds died 2 or 3 days after completion of dosing with PAD and on post mortem examination each of the four birds exhibited severe liver damage. Since PAD was given in isopropanol solution, it was highly probable that the isopropanol was the cause of death. PAD alone has been reported to produce signs similar to those

TABLE 1. EFFECT OF P2S, PAD AND ATROPINE UPON THE DEVELOPMENT OF DELAYED NEUROTOXIC SIGNS IN CHICKENS POISONED WITH DFP

Dose of DFP mg/kg	Incidence				Severity				Latent period (days)			
	No treatment	P2S + atropine	P2S + PAD + atropine	P2S + atropine + PAD (5 daily doses)	No treatment	P2S + atropine	P2S + PAD + atropine	P2S + atropine + PAD (5 daily doses)	No treatment	P2S + atropine	P2S + PAD + atropine	P2S + atropine + PAD (5 daily doses
0·2	0/5	0/10	0/5	0/4	0	0	0	0	0	0	0	0
0·3	3/5	3/4			2·6	3·0			10·7	11·0		
0·4	4/5	6/10	2/5	4/4	4·0	2·9	3·2	5·0	10·5	10·2	9·5	8·8
0·5	1/2	5/5			4·0	6·8			10·0	9·2		
0·6		10/10	4/5	3/4		4·5	6·4	7·8		10·2	9·0	8·2
0·7		5/5				3·8				9·4		
0·8		9/10	4/4	4/4		6·8	7·5	7·0		9·5	8·0	8·2
0·9		5/5				7·4				9·0		

Oximes and atropine were administered i.m. 10 min before i.m. DFP.

The "severity score" is the mean of the markings for each bird in the group. The latent period is the mean of the intervals from dosing to the day neurotoxic signs were obvious to both observers.

produced by TOCP. These were ataxic gait, rigidity of neck muscles and an exaggerated curvature of the neck simulating an overfilled crop. These signs could affect the interpretation of border line cases; consequently they were carefully looked for but none were seen.

The effects of prior treatment with oximes and atropine upon the development of delayed neurotoxic signs produced by DFP. Table 1 shows how the incidence, severity and latent period to development of neurotoxic signs are influenced by the administration of oximes and atropine. The results may be summarized as follows:

(1) P2S and atropine do not influence the incidence, severity or the time to onset of neurotoxic signs in chickens poisoned with DFP.

(2) The addition of PAD to this mixture, whether it is given as a single dose together with P2S and atropine prior to poisoning or whether it is given, in addition to the above, on 4 consecutive days after poisoning also does not affect the development of ataxia and paralysis.

(3) The minimum intramuscular dose of DFP necessary to produce delayed neurotoxicity is 300 μg/kg.

TABLE 2. THE CUMULATIVE EFFECT OF DFP

		Time (days) from first injection							
Bird no.		19	20	21	22	23	24	25	26
53	Severity score per bird	2	3	2		2	2	2	3
54		4	5	4	5	6	7	8	8
55		1	2	2		2	1	1	2
56		2	4	4		4	3	4	3
57		0	0	1	0	0	2	1	3
58		3	4	4	2	3	5	6	7
Mean severity score		2	3	3	$2\frac{1}{2}$	3	$3\frac{1}{3}$	$3\frac{2}{3}$	$4\frac{1}{3}$

100 μg/kg i.m. on days 0, 3, 6, 9, 12 and 15. No oximes or atropine given.

The cumulative effects of subneurotoxic doses of DFP and sarin. Table 2 shows the progressive severity of neurotoxic signs on successive days after completion of dosing with DFP in unprotected chickens. DFP 100 μg/kg was given intramuscularly at 3 day intervals such that on the 15th day after commencement of dosing each bird had received 6 $\times$ 100 μg of DFP. Nineteen days after the first injection five of the six birds exhibited definite signs of ataxia and on the 21st day all birds were affected. If the latent period before onset of signs is taken as 10 days, then birds 53, 54, 56 and 58 had received a neurotoxic dose by day 9, i.e. after it had received 4 doses of 100 μg/kg. This finding was confirmed in a subsequent experiment when only 4 doses of

100 μg/kg were given at 3 day intervals and each bird, so dosed, showed definite signs of neurotoxicity 10 days after the fourth and final dose. Table 1 showed that the minimum dose of DFP required to produce neurotoxicity was 300 μg/kg.

This type of experiment was repeated with Sarin. Preliminary experiments showed that the intramuscular LD50 of Sarin for chickens was 25 μg/kg and that in protected birds the single, minimum, intramuscular dose necessary to produce ataxia was 650–760 μg/kg i.e. 26–30 LD_{50}.

Ten birds were given 25 μg/kg Sarin intramuscularly daily for several days. Although the birds were protected with P2S and atropine they all exhibited convulsions which lasted for a short time after each of the first few doses. During this period the cumulative acute effects on the birds were severe and in some cases dosing had to be delayed for 24 hr until they had recovered. However, with successive doses the acute effects became less severe and after a fortnight individual birds were only mildly affected by an LD_{50} dose.

Their general condition deteriorated during the course of the experiment and at its conclusion loss in weight of up to 50 per cent of the pre-experimental level was observed in many birds.

Of the 10 birds which started the experiment, one died after the 7th dose and another was so severely affected by the acute effects that dosing had to be stopped after 11 doses.

The remaining 8 birds received 26–28 doses, i.e. 26–28 $\times$ LD_{50}. Five showed slight but definite ataxia at the end of the experiment, but the remaining three had no abnormalities of gait.

Three of the above birds were examined histologically. The first bird received only 11 $\times$ LD_{50} Sarin. It exhibited no signs of ataxia, nevertheless the sciatic nerve contained some degenerating fibres as did the spinal cord and medulla.

Of the two remaining birds one which received 29 $\times$ LD_{50} showed no abnormal gait or movements. There are, however, numerous degenerating fibres in the sciatic nerve

TABLE 3. EFFECT OF INTERVAL BETWEEN THE INTRAMUSCULAR ADMINISTRATION OF SUB-NEUROTOXIC DOSES OF DFP UPON THE DEVELOPMENT OF DELAYED NEUROTOXIC SIGNS

Dose μg/kg			Neurotoxic signs		
1st	2nd	Interval between doses (days)	Incidence	Interval from 2nd dose to onset of signs	Mean severity of signs
150			0/5		0
200			0/5		0
350			4/4	10	5·2
200	150	1	5/5	14	3·6
200	150	4	5/5	14	3·4
200	150	8	5/5	10	4·4
200	150	10	4/5	10	2·6
200	150	16	3/5	10	3·0

together with similar but less extensive damage in the posterior dorso-lateral and ventral columns of the spinal cord and medulla.

The 3rd chicken received 28 × LD_{50} dose and exhibited gross ataxia with extensive degeneration in numerous fibres in the sciatic nerve. Damage was generalized but less severe in many of the fibres in the spino cerebellar and ventral tracts in the spinal cord.

Effect of interval between the intramuscular administration of two sub-neurotoxic incapacitating doses of DFP. Table 3 shows the effect of varying the interval between the intramuscular administration of two sub-incapacitating doses of DFP on the production of neurotoxicity.

Neither 150 nor 200 μg/kg when given alone, produced any signs of incapacitation, whereas 350 μg/kg alone produced marked delayed neurotoxic effects in all the birds examined. A dose of 200 μg/kg followed by 150 μg/kg gave rise to quite definite signs of ataxia even though an interval as long as 16 days had elapsed between administering the doses.

DISCUSSION

The phosphono- and phosphorofluoridates are lethal at very low doses, but administration of oxime and atropine enables animals to tolerate many times the LD_{50} dose. Many of these substances are also neurotoxic and an induced tolerance to lethal doses by the use of antidotes, such as the above, may predispose the animal to delayed neurotoxic effects.

Henschler[2] claimed that the fat soluble oxime PAD was effective therapeutically in TOCP poisoning. He also showed that when 100 mg/kg PAD was given 6–10 hr after intoxication followed by similar doses daily on 6 successive days, the minimum paralysing dose of TOCP in chickens was increased by a factor of 3 or 4 and the onset of signs was delayed. When a total dose of 700 mg/kg of PAD was given under these circumstances it produced reversible spastic signs. This was not seen in our experiments possibly due to the fact that only 500 mg/kg was used. The action of PAD might well have been to delay the rate of absorption of TOCP from the gut and so increase the dose necessary to produce paralysis.

The significance of the inhibition of cholinesterase in the production of delayed neurotoxic effects has been discussed elsewhere.[6] It is, however, unlikely that this process is the one involved for the following reasons: (a) it is possible to inhibit the cholinesterase of the central nervous system without producing neurotoxic signs,[7] (b) not all anticholinesterases are neurotoxic,[1] (c) tri-paraethylphenyl phosphate which is a potent neurotoxic compound does not inhibit cholinesterase either *in vivo* or *in vitro*,[8] (d) P2S which is a potent reactivator of phosphorylated cholinesterase and therapeutically effective against the lethal effects of organo–phosphorus compounds has no effect on the onset or severity of DFP-induced delayed neurotoxicity. This has been supported by Johnson[9,10] who has shown that organo–phosphorus anticholinesterase compounds are capable of inhibiting other esterases in the chicken central nervous system in addition to cholinesterase. Those compounds which produce a delayed neurotoxic effect phosphorylate a specific esterase site in the brain soon after administration and the resultant toxic effect is due not to the loss of esterase activity per se but to loss of some other function of the esterase protein molecule when it is phosphorylated.

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