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Quantitative studies of the effect of antagonists on the acute toxicity of organophosphates in rats

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Summary

1. The subcutaneous acute toxicities of the vinyl phosphate pesticides monocrotophos, dicrotophos, chlorfenvinphos, crotoxyphos, dichlorvos, mevinphos, and of the experimental compounds SD 4455 (*cis*-2-carboxy-1-methylvinyl dimethylphosphate) and SD 7779 (*cis*-2-(1-phenylethoxy) carbonyl-1-methylvinyl diethylphosphate) have been determined in female rats.

2. The effects on the log dose-probit mortality curves to the vinylphosphates of the therapeutic subcutaneous administration of methylatropine, atropine, *N*-methylpyridinium-2-aldoxime methanesulphonate and obidoxime have been studied.

3. Elevation of the LD50 values by the therapeutic regimens was shown to be an unsatisfactory measure of therapeutic efficiency, while reduction of the effect of a maximally lethal dose (LD90) to less than that of a minimally lethal dose (LD10) provided a better quantitative measure of therapeutic efficiency.

4. The combination of atropine sulphate (50 μmol base/kg) with obidoxime (250 μmol/kg) was found to be generally the most effective of the antidotal regimens.

Introduction

The symptoms of acute intoxication of animals by many organophosphorus compounds may be attributed primarily to inhibition of acetylcholinesterase at effector organs and synapses supplied by cholinergic nerve fibres. Whereas atropine blocks some of the more severe effects of intoxication, nucleophilic attack of the phosphorylated enzyme by *N*-methylpyridinium aldoximes (Wilson & Ginsburg, 1955 ; Childs, Davies, Green & Rutland, 1955) before " ageing " occurs (Hobbiger, 1955 ; 1956 ; Jandorf, Michel, Schaffer, Egan & Summerson, 1955) results in the reactivation of acetylcholinesterase, and treats the cause, rather than the effect of the intoxication. A combined therapy of atropine with oximes is generally more efficient than treatment by either of the two antidotes alone (see, for example, Kewitz & Wilson, 1956 ; Davies & Green, 1959 ; Hobbiger, 1963).

Organophosphorus cholinesterase inhibitors are designated as either " oxime sensitive " or " not oxime sensitive ", depending on the survival of groups of rats or guinea-pigs when injected with $5 \times$ LD50 of these inhibitors followed by the therapeutic injection of an oxime and atropine sulphate (Working Document No. 3, Pesticides Safety Precautions Scheme, Ministry of Agriculture, Fisheries & Food, 1966). This type of " all-or-none " study does not give a quantitative assessment of the efficacy of such antidotes against organophosphate intoxication.

The following paper describes investigations in which a quantitative assessment has been made in rats of the efficacy of therapeutic measures against the acute lethal effects of organophosphorus cholinesterase inhibitors, and examines the influence of various antidotal regimens on the mortality curves of these compounds. A similar study in mice has been reported previously by Parkes & Sacra (1954).

Methods

Female rats, weighing between 200 and 250 g, of the Carworth Farm E strain and bred under SPF conditions in these laboratories, were used throughout. All compounds, both organophosphates and antidotes, were administered by subcutaneous injection in the flank.

The organophosphates were either dissolved or suspended by ultrasonic agitation in physiological saline and administered in a dose volume of 1 ml/kg. For chlorfenvinphos, ultrasonic agitation was used and the vehicle incorporated Acacia Powder B.P., 5% w/v, to improve the stability of the resulting suspension. Solutions of atropine sulphate and atropine methonitrate were prepared in physiological saline, while those of the oximes were prepared in distilled water, to provide a dose-volume of 1 ml/kg. The doses of antidotes routinely used were: atropine methonitrate (methylatropine), 18·02 mg/kg; atropine sulphate (atropine), 17·4 mg/kg (both equivalent to 50 μmol base/kg); *N*-methylpyridine-2-aldoxime methanesulphonate (P2S), 50 mg/kg, and *bis*-(4-hydroxyiminomethyl pyridinium-1-methyl) ether dichloride (obidoxime), 90 mg/kg (both oximes equivalent to 250 μmol/kg). When atropine and either P2S or obidoxime were to be given simultaneously, the respective solutions were mixed in equal quantities, and the resulting solution injected in a dose-volume of 2 ml/kg.

The median lethal doses (LD50) of the different organophosphorus compounds, corrected for active material (that is, the proportion of the original sample having insecticidal activity), were estimated in groups of five rats with or without the administration of the antidotes. Between five and seven logarithmically spaced dose increments of the organophosphates were used in each determination of the mortality curves. These increments depended on the slopes of the curves and the dose ratios varied from 1·095 for steep curves to 1·50 for the more shallow curves.

The antidotes were injected in the flank opposite to that in which the organophosphate had been injected, when the first signs of intoxication became apparent (for example, salivation). In the case of monocrotophos, dicrotophos, dichlorvos, mevinphos and SD 4455 the antidotes were administered soon after the organophosphate because of the rapid rate of onset of the symptoms of intoxication. In the case of the remaining organophosphates, the antidotes were administered only when the symptoms appeared, with a maximum delay of 60 min in the case of chlorfenvinphos. The effect of antidotes in organophosphate intoxication depends on the timing of administration, and has been shown to be most beneficial if delayed until the onset of the effects of intoxication (Sanderson, 1965). Each dose group of five rats was housed in a single cage measuring $36 \times 23 \times 17$ cm and mortalities were recorded over a period of 7 days. The LD10, LD50 and LD90 values were estimated by the probit method for each compound and each antidotal schedule. The slopes of the log dose-probit mortality lines were calculated by the method

of least squares. Parallelism between the log dose-probit mortality lines of the untreated and treated groups was examined by analysis of chi-squared. Probit analysis (Finney, 1962) and statistical calculations were performed with a computer.

Compounds

The organophosphates examined were monocrotophos (*cis**-2-methylcarbamoyl-1-methylvinyl dimethylphosphate, analytically pure material), dicrotophos (*cis**-2-dimethylcarbamoyl-1-methylvinyl dimethylphosphate, 92% active material), chlorfenvinphos (beta†-2-chloro-1-(2′,4′-dichlorophenyl) vinyl diethylphosphate, 92% active material), crotoxyphos (*cis**-2-(1-phenylethoxy) carbonyl-1-methylvinyl dimethylphosphate, 86% active material), dichlorvos (2,2-dichlorovinyl dimethylphosphate, 94% active material), and mevinphos (*cis**-2-methoxycarbonyl-1-methylvinyl dimethylphosphate, 98·6% active material). These vinylphosphates are products of the Shell International Chemical Company. SD 4455 (*cis**-2-carboxy-1-methylvinyl dimethylphosphate, analytically pure material) and SD 7779 (*cis**-2-(1-phenylethoxy) carbonyl-1-methylvinyl diethylphosphate, analytically pure material) were supplied by Shell Development Company, Modesto, California.

The antidotes examined were atropine sulphate, B.P. (John Bell, Hills & Lucas, Ltd.), atropine methonitrate, B.P. (Burroughs Wellcome & Co.) and P2S (*N*-methylpyridinium-2-aldoxime methanesulphonate) (Koch-Light). Obidoxime (*bis*-(4-hydroxyiminomethyl pyridinium-1-methyl) ether dichloride, Toxogonin) was generously supplied by E. Merck (Darmstadt). The structural formulae of the organophosphates and oximes used in this study are shown in Fig. 1.

Results

The estimated subcutaneous LD50 values of the organophosphates alone and in the presence of the different therapies are shown in Table 1. In the majority of cases, the therapeutic administration of the antidotes resulted in a statistically significant elevation of the LD50 values.

The slopes of the log dose-probit mortality curves for the organophosphates in the presence and the absence of the different antidotal regimens are presented in Table 2. In the case of monocrotophos, dicrotophos, SD 7779, crotoxyphos and chlorfenvinphos there was no significant deviation from parallelism between the log dose-mortality slopes in animals receiving only the organophosphate and those in animals also receiving antidotal treatment. Significant reductions in the slopes of the log dose-mortality curves of mevinphos were produced by treatment with P2S and with atropine/obidoxime, of dichlorvos with atropine/P2S, and of SD 44[5]5 with atropine or obidoxime. Large differences between the slopes of the log dose-mortality curves of individual organophosphates in the absence of any therapeutic regimen are apparent.

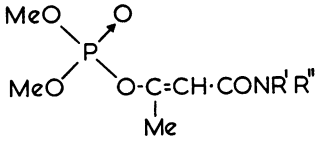
An alternative means of expressing the decrease in the toxicity of the organophosphorus cholinesterase inhibitors by antidotal regimens is presented in Table 3, which lists the LD10 (minimally lethal dose) and LD90 (maximally lethal dose) values in the absence and in the presence of the therapies. In each case, except that

* *cis* refers to the configuration of the crotonic acid from which these compounds are synthesized.

† *beta* refers to the *cis* configuration of the chlorine atoms and the phosphorus-containing group.

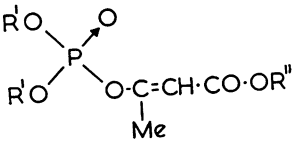
of crotoxyphos, the combination of obidoxime (250 μmol/kg) with atropine (50 μmol base/kg) moves the log dose-mortality curve to the right, so that a dose producing an estimated 90% mortality in rats not receiving the therapy is not lethal to 10% of the animals which receive this therapy. This is evident as the LD10 in treated animals is significantly greater than the LD90 in untreated animals. The combination of P2S with atropine similarly significantly raises the LD10 of monocrotophos, dicrotophos, dichlorvos, SD 4455 and SD 7779 above the LD90 values in untreated animals. Atropine alone demonstrated this effect against monocrotophos, dicrotophos and SD 4455, and obidoxime alone against monocrotophos, SD 4455 and SD 7779. Methylatropine alone was significantly effective against monocrotophos only. Thus, organophosphate intoxication in rats is best treated in the majority of cases by atropine (50 μmol base/kg subcutaneously) in combination with obidoxime (250 μmol/kg subcutaneously).

Organophosphates



R′=Me R″=H Monocrotophos

R′=Me R″=Me Dicrotophos

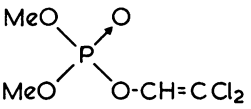


R′=Me R″=H SD 4455

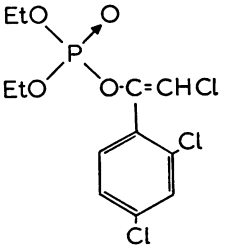
R′=Me R″=Me Mevinphos

R′=Me R″=CHMe(Ph) Crotoxyphos

R′=Et R″=CHMe(Ph) SD 7779

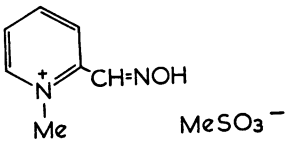


Dichlorvos

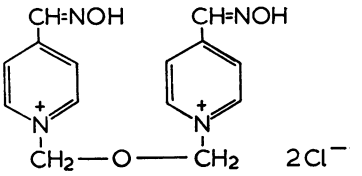


Chlorfenvinphos

Oximes



N-methylpyridine-2-aldoxime methanesulphonate (P2S)



Obidoxime

FIG. 1

TABLE 1. *Effect of antidotal regimens on median lethal doses of organophosphates following subcutaneous injection in female rats*

Organophosphate	Untreated	Methylatropine 50 μmol base/kg	Atropine 50 μmol base/kg	P2S 250 μmol/kg	Atropine with P2S	Obidoxime 250 μmol/kg	Atropine with Obidoxime
Monocrotophos	31·2 (28·6–34·1)	94·6* (81·9–110·5)	365·1* (299·5–440·5)	81·8* (69·2–101·4)	890·1* (739·8–1,234·0)	146·3* (122·5–176·4)	730·4* (591·4–900·4)
Dicrotophos	34·3 (29·1–40·6)	78·6 (N.E)	250·9* (225·3–274·7)	93·7* (79·8–111·4)	249·8* (205·5–298·4)	141·9* (113·7–176·4)	345·8* (294·4–405·1)
Chlorfenvinphos	43·4 (34·4–53·5)	61·8 (43·2–82·0)	104·1* (82·3–131·8)	54·1 (44·4–65·2)	216·0* (170·2–274·0)	87·2* (72·9–101·8)	800·3* (670·3–965·2)
Crotoxyphos	148·8 (124·5–179·4)	223·3* (191·6–259·6)	298·3* (256·4–346·6)	239·3* (203·9–285·4)	278·8* (238·6–321·7)	207·0 (166·9–244·7)	257·3* (219·9–305·6)
Dichlorvos	52·3 (44·5–61·5)	64·1 (54·2–75·8)	176·6* (145·3–214·8)	258·6* (188·3–356·5)	939·8* (648·7–1,411·0)	359·5* (259·6–497·0)	1,212·0* (866·0–1,696·0)
Mevinphos	5·4 (5·0–5·8)	5·8 (5·0–6·7)	8·3* (7·1–9·9)	9·3* (7·6–12·2)	10·4* (8·9–12·1)	10·8* (9·0–12·7)	23·8* (21·7–27·1)
SD 4455	147·4 (139·3–155·8)	242·7* (224·1–260·8)	1,546·0* (1,255·0–1,873·0)	238·0* (213·7–259·2)	None died at 589·7	466·6* (374·8–533·5)	None died at 849·2
SD 7779	11·4 (9·6–13·5)	25·8* (21·8–30·6)	46·2* (39·9–53·2)	39·8* (34·4–46·0)	61·1* (56·0–66·2)	95·4* (80·9–110·0)	147·5* (125·0–175·3)

* Significantly different from untreated (control) group ($P<0·05$). N.E., Data did not allow estimation of fiducial limits.
Five animals were used at each dose level, and the median lethal doses (LD50 values) are given as μmol active material/kg, with 95% fiducial limits in parentheses.

TABLE 2. *Slope values* (±S.E.) *of log dose-probit mortality curves for subcutaneously injected organophosphates in female rats*

Organophosphate	Untreated	Methylatropine 50 μmol base/kg	Atropine 50 μmol base/kg	P2S 250 μmol/kg	Atropine with P2S	Obidoxime 250 μmol/kg	Atropine with Obidoxime
Monocrotophos	14·7±3·5	17·3±6·5	9·3±3·1	9·9±3·4	7·2±2·5	8·7±2·6	7·3±3·0
Dicrotophos	12·0±3·9	47·8±30·6	15·9±5·1	15·5±5·8	10·1±3·3	8·2±2·3	17·1±6·4
Chlorfenvinphos	6·2±1·8	4·5±1·6	6·6±2·2	9·3±3·1	5·2±1·4	11·7±3·9	8·7±2·6
Crotoxyphos	8·7±2·6	13·2±4·3	15·7±5·6	11·2±3·7	17·3±6·4	9·9±3·4	13·8±5·0
Dichlorvos	20·6±8·4	14·1±5·0	8·2±2·4	8·5±3·2	4·2*±1·3	10·3±4·2	7·0±2·5
Mevinphos	25·3±8·2	19·1±7·2	11·2±3·7	6·7*±2·2	15·6±5·6	11·3±3·7	6·6*±1·6
SD 4455	17·2±3·3	12·4±3·0	7·8*±2·4	13·3±3·4	N.E	9·0*±2·7	N.E
SD 7779	10·9±3·5	11·0±3·6	19·0±7·1	22·8±9·2	22·4±7·3	18·5±7·1	9·7±2·9

* Significantly different from untreated (control) group ($P<0·05$). N.E., Data did not allow estimation of slope value.

TABLE 3. *Effect of antidotal regimens on LD10 and LD90 values of organophosphates following subcutaneous injection in female rats*

Organophosphate		Untreated	Methylatropine 50 μmol base/kg	Atropine 50 μmol base/kg	P2S 250 μmol/kg	Atropine with P2S	Obidoxime 250 μmol/kg	Atropine with Obidoxime
Monocrotophos	LD10	25·5 (20·8–28·0)	79·7 (48·9–89·0)	266·0 (135·9–316·8)	60·8 (34·1–71·2)	591·0 (291·2–717·0)	104·2 (62·1–124·0)	488·0 (242·8–599·5)
	LD90	38·1 (34·7–46·6)	112·3 (100·2–187·4)	501·1 (422·2–957·5)	110·1 (92·5–219·7)	1,340·0 (1,051·0–3,796·0)	205·4 (171·9–353·3)	1,093·0 (890·4–2,189·0)
Dicrotophos	LD10	26·8 (16·8–31·0)	73·9 (N.E.)	208·5 (145·8–230·2)	77·5 (45·1–87·6)	186·6 (103·7–220·7)	99·3 (46·4–120·8)	291·0 (171·5–326·8)
	LD90	43·9 (38·0–70·5)	83·6 (N.E.)	302·0 (275·6–414·0)	113·3 (100·0–200·4)	334·5 (284·6–577·3)	203·0 (167·0–429·6)	411·1 (366·2–693·1)
Chlorfenvinphos	LD10	27·0 (13·1–34·2)	32·3 (6·9–45·2)	66·4 (27·6–83·6)	39·4 (20·1–46·9)	122·4 (58·2–158·8)	67·8 (39·6–78·7)	570·0 (339·6–678·1)
	LD90	69·7 (55·8–135·4)	118·3 (87·0–462·3)	163·3 (128·8–391·8)	74·2 (62·5–141·7)	381·2 (293·7–801·0)	112·2 (97·5–181·6)	1,124·0 (940·2–1,933·0)
Crotoxyphos	LD10	106·0 (62·9–126·1)	178·4 (116·2–203·5)	247·0 (152·3–278·1)	184·0 (112·6–212·8)	235·1 (141·1–263·1)	153·8 (77·1–183·0)	207·9 (120·8–235·3)
	LD90	208·9 (174·8–360·1)	279·4 (245·1–430·4)	360·2 (320·1–582·7)	311·3 (267·6–528·4)	330·7 (296·3–535·7)	278·5 (237·8–497·4)	318·4 (279·1–566·6)
Dichlorvos	LD10	45·3 (25·0–50·3)	51·9 (30·3–59·3)	123·3 (70·9–148·7)	182·8 (64·6–230·6)	466·5 (162·3–669·4)	270·1 (80·3–331·8)	795·8 (271·7–1,036·0)
	LD90	60·4 (54·5–109·7)	79·1 (69·3–135·5)	253·1 (209·8–440·2)	365·9 (289·9–1,044·0)	1,893·0 (1,298·0–5,935·0)	478·5 (389·6–1,604·0)	1,845·0 (1,417·0–5,409·0)
Mevinphos	LD10	4·8 (3·8–5·1)	5·0 (3·1–5·5)	6·4 (3·9–7·4)	6·0 (2·7–7·4)	8·6 (5·3–9·7)	8·3 (4·9–9·6)	15·2 (10·4–17·6)
	LD90	6·0 (5·6–7·6)	6·8 (6·1–10·9)	10·8 (9·3–18·4)	14·6 (11·5–37·1)	12·6 (11·1–20·3)	14·0 (12·1–22·9)	37·2 (31·0–60·9)
SD 4455	LD10	124·1 (109·5–132·6)	191·3 (150·1–211·0)	1,057·0 (551·0–1,289·0)	190·6 (144·5–212·6)	>589·7	322·1 (197·0–381·6)	>849·2
	LD90	175·1 (164·0–197·8)	307·9 (280·9–384·3)	2261·0 (1,868·0–4,159·0)	297·3 (270·6–369·1)	>589·7	619·1 (521·9–1,020·0)	>849·2
SD 7779	LD10	8·7 (5·2–10·1)	19·7 (11·9–22·9)	39·5 (24·5–43·9)	35·0 (20·6–38·4)	53·6 (41·1–57·8)	81·3 (46·7–90·8)	108·8 (68·7–127·7)
	LD90	15·0 (12·9–24·8)	33·7 (29·0–56·6)	53·9 (48·6–83·4)	45·3 (41·3–77·1)	69·7 (64·8–89·1)	111·9 (100·7–185·4)	200·0 (170·0–321·7)

N.E., Data did not allow estimation of fiducial limits.
Results are expressed in μmol active material/kg with 95% fiducial limits in parentheses.

Table 4 presents a summary of the efficacies of the antidotal treatments assessed by the two criteria: (*a*) elevation of the LD50 values (obtained by dividing the LD50 value in the presence of therapy by that in non-treated animals) and (*b*) separation of the overlap of the mortality curves in the absence and presence of therapy (obtained by dividing the LD10 in treated animals by the LD90 in untreated animals). Discrepancies are evident between these two criteria. Whereas all the treatments except obidoxime alone significantly elevated the LD50 of crotoxyphos, for example, none raised the minimally lethal dose in the presence of therapy above the maximally lethal dose in the absence of therapy. Treatment with methylatropine significantly raised the LD50 values of monocrotophos, crotoxyphos, SD 4455 and SD 7779, yet only in the case of monocrotophos was the LD10 in treated animals significantly elevated above the LD90 in untreated animals. The table shows that separation of the overlap of the mortality curves in the absence and presence of therapy is a more meaningful criterion of therapeutic efficacy than is elevation of the LD50.

Discussion

The acute toxicity of organophosphates in rats has been measured following subcutaneous injection, both in the absence and presence of therapeutic regimens. The efficacy of these regimens in reducing the toxicity of the organophosphates has been examined using two criteria: (i) the elevation of the LD50 value; (ii) the difference between the LD90 dose in the absence of therapy and the LD10 dose in the presence of therapy.

TABLE 4. *Effectiveness of antidotal regimens in reducing the toxicity of organophosphates*

(*a*) *By elevation of the LD50 value* (results expressed as $\frac{\text{LD50 (treated)}}{\text{LD50 (untreated)}}$)

Organophosphate	Methyl-atropine 50 μmol base/kg	Atropine 50 μmol base/kg	P2S 250 μmol/kg	Atropine with P2S	Obidoxime 250 μmol/kg	Atropine with Obidoxime
Monocrotophos	3·0*	11·7*	2·6*	28·5*	4·7*	23·4*
Dicrotophos	2·3	7·3*	2·7*	7·3*	4·1*	10·1*
Chlorfenvinphos	1·4	2·4*	1·2	5·0*	2·0*	18·4*
Crotoxyphos	1·5*	2·0*	1·6*	1·9*	1·4	1·7*
Dichlorvos	1·2	3·3*	4·9*	17·6*	6·7*	22·7*
Mevinphos	1·1	1·5*	1·7*	1·9*	2·0*	4·4*
SD 4455	1·6*	10·5*	1·6*	>4	3·0*	>6
SD 7779	2·3*	4·1*	3·5*	5·4*	8·4*	12·9*

(*b*) *By raising the LD10 in animals receiving therapy above the LD90 in animals not receiving therapy* (results expressed as $\frac{\text{LD10 (treated)}}{\text{LD90 (untreated)}}$)

Organophosphate	Methyl-atropine 50 μmol base/kg	Atropine 50 μmol base/kg	P2S 250 μmol/kg	Atropine with P2S	Obidoxime 250 μmol/kg	Atropine with Obidoxime
Monocrotophos	2·1*	7·0*	1·6	15·5*	2·7*	12·8*
Dicrotophos	1·7	4·7*	1·8	4·3	2·3	6·6
Chlorfenvinphos	0·5	1·0	0·6	1·8	1·0	8·2*
Crotoxyphos	0·9	1·2	0·9	1·1	0·7	1·0
Dichlorvos	0·9	2·0	3·0	7·7*	4·5	13·2*
Mevinphos	0·8	1·1	1·0	1·4	1·4	2·5*
SD 4455	1·1	6·0	1·1	>3·4	1·8	>4·9
SD 7779	1·3	2·6	2·3	3·6*	5·4*	7·3*

* Significantly elevated above untreated value ($P<0·05$).

Elevation of the LD50 of a toxic compound by a therapeutic regimen measures the degree of shift of the mortality curve to the right along the abscissa, assuming the slopes of the mortality curves in untreated and therapeutically treated animals are parallel. If the mortality curves are steep, a significant elevation of the LD50 should move the curve so far to the right that the maximally lethal dose now exerts no lethal effect in the presence of the therapy. If the curve is shallow, however, a significant elevation of the LD50 may only reduce the effect of the maximally lethal dose without making it ineffective ; that is some of the rats of the population studied will still die.

The speed at which death ensues from massive exposure to organophosphates is high, and in most cases therapy will be of no avail. However, the conditions most likely to be encountered in clinical practice are those in which the patient is not moribund following exposure to an organophosphate, but is presenting symptoms of intoxication. It is at this stage of exposure that the patient is most likely to respond to therapy. Instances have been reported in which recovery of unconscious patients from very large exposures to the phosphorothionate parathion has been achieved, using atropine and obidoxime therapy over a prolonged period (Barckow, Neuhaus & Erdmann, 1969). The rate of conversion of parathion to its active metabolite, paraoxon, is of a low order (Kubištova, 1959), which would account for this success.

The object of treating intoxication by any lethal compound is to reduce the effect of a maximally active dose to below that of a minimally active dose. The use of a computer programme for probit analysis in this study has measured these values for each organophosphate in the absence and presence of therapies. In order to apply fiducial limits to these values at the 95% probability level, the maximally lethal dose was taken as the LD90, and the minimally lethal dose as the LD10. Significant elevation of the LD10 in treated animals above the LD90 in untreated animals indicates complete protection of all animals against the lethal effects of a dose of the organophosphate ($P<0{\cdot}05$) that would otherwise be fatal.

The therapeutic regimens applied to intoxicated rats resulted in a shift of the log dose-probit mortality curves to the right. In general, there was no statistically significant deviation from parallelism in these curves from those of the intoxicated animals not receiving therapy. However, in those instances where statistically significant differences occurred, they were evident as decreased slope values. This observation agrees with the suggestion of Davies, Green & Willey (1959) that the slopes of the mortality curves almost always decrease as the treatment of organophosphate poisoning becomes increasingly effective.

The two criteria of assessment of efficacy yield conflicting results. All the therapies, except obidoxime alone, significantly elevated the LD50 of crotoxyphos, yet none reduced the effect of an LD90 dose below that of an LD10 dose. It is this latter quality of the treatment which is desirable therapeutically.

The concept of describing organophosphates as " oxime sensitive " has depended on the ability of the combination of fixed doses of atropine and an oxime to protect all animals (rats or guinea-pigs) of a group from death due to five times the LD50 of any organophosphate (Working Document No. 3, Pesticides Safety Precautions Scheme, Ministry of Agriculture, Fisheries & Food, 1966). The variation in the slope values between the mortality curves of the different organophosphates indicates

that a dose of five times the LD50 of a compound with a steep mortality curve would exceed the LD90 to a greater extent than that for a compound with a more shallow slope to its mortality curve. Mevinphos yields a log dose-probit mortality curve having an estimated mean slope value of 25·3. The LD50 is 5·4 μmol/kg subcutaneously and 5 × LD50 is therefore 27 μmol/kg subcutaneously, which is 4·5 × its LD90 value of 6·0 μmol/kg. Chlorfenvinphos yields a mortality curve with a slope of 6·2, and has an LD50 of 43·4 μmol/kg subcutaneously ; 5 × LD50 is therefore 217·0 μmol/kg, which is only 3·1 × the LD90 of 69·7 μmol/kg. It is therefore unjustified to adopt multiples of the LD50 as a unit of dose and the work presented shows that the reduction of the effects of a maximally lethal dose of a compound below those of a minimally lethal dose provides better means for expressing protection quantitatively.

Although none of the therapeutic antidotal regimens significantly raised the LD10 value for crotoxyphos above the LD90 value in untreated animals, a combination of atropine with either P2S or obidoxime brought about this separation with its chemical congeners mevinphos, SD 4455 and SD 7779. Since both P2S and obidoxime are able to reactivate the cholinesterase activities of rat brain homogenates *in vitro* and of guinea-pig whole blood *in vivo* following inhibition with crotoxyphos (Natoff & Reiff, unpublished observations) the reason for the low order of response of intoxication by crotoxyphos to therapy is being studied further.

Parkes & Sacra (1954) showed the acute toxicities of some cholinesterase inhibitors to be reduced by the simultaneous administration of atropine, hexamethonium or (+)-tubocurarine, either alone or in combination, following intravenous injection in mice. Two criteria of activity were adopted in this study (i) elevation of the LD50 in the presence of the treatment compared with that in its absence ; (ii) estimation of the dose of the acetylcholine antagonist(s) which reduces the effect of a dose of the cholinesterase inhibitor from that which kills 80% of the mice to one which kills 40%. They found that as the LD50 increased with the therapies so the slopes of the mortality curves also increased in a majority of instances, in contrast to the suggestion of Davies *et al.* (1959) and the findings in the present study. In attempting to speculate on the principal sites of action of cholinesterase inhibitors from the protection afforded by the acetylcholine antagonists, Parkes & Sacra (1954) acknowledge the lack of absolute specificity of these agents for their recognized loci of activity. In the present study, differences in the therapeutic activity between equimolar amounts of methylatropine and atropine are evident which tempt speculation as to the effects of the organophosphates at peripheral and central cholinoceptor regions, but the doses used are too high to allow any specificity of locus of action to be attributed to these blocking agents.

Wherever the combination of atropine and an oxime resulted in an enhanced therapeutic activity, obidoxime was more effective than P2S on a molar basis. The superiority of the combination of obidoxime with atropine over that of P2S with atropine in the treatment of experimental intoxication by organophosphates also applies to the protection of mice against intoxication by dichlorvos (Jaques, 1964) and of rats and guinea-pigs against intoxication by sarin (Fleisher, Harris, Miller, Thomas & Cliff, 1970). Whereas P2S has only one hydroxyimino (aldoxime) group per molecule, obidoxime has two. It may be argued therefore that quantitative comparisons of the two oximes may be carried out on the basis of equivalence and not of molarity. The latter procedure was adopted, however, because Bieger &

Wassermann (1967) showed that one of the hydroxyimino groups of obidoxime is 41·8% dissociated to the active anion (Wilson, Ginsburg & Quan, 1958) at pH 7·4, while the second group is only 12·7% dissociated. For *N,N'*-trimethylene-*bis*-(pyridinium-4-aldoxime) dibromide (TMB-4) these values are 29·5% and 5·8% at pH 7·4. Thus, the contribution of the second hydroxyimino group to the reactivating activity would appear to be minimal. In spite of the higher degree of dissociation of obidoxime to the active anion, Hobbiger & Vojvodić (1966) found TMB-4 to be marginally more potent than obidoxime in reactivating the diethylphosphoryl and the di-*iso*propylphosphoryl acetylcholinesterase of washed human erythrocytes at pH 7·45.

Hobbiger, Pitman & Sadler (1960) showed TMB-4 to have 22 times the reactivating potency of *N*-methylpyridinium-2-aldoxime iodide (P-2-AM) against the diethylphosphoryl acetylcholinesterase of human erythrocytes. This ratio was 30 against the diethylphosphoryl acetylcholinesterase of bovine erythrocytes. Using di-*iso*-propylphosphoryl acetylcholinesterase of human erythrocytes, these workers found TMB-4 to have 52 times the reactivating potency of P-2-AM. Thus, the greater therapeutic effect of obidoxime over an equimolar amount of P2S against intoxication by dialkylphosphates demonstrated in this work may be explained by the greater reactivating potency of the *bis*-pyridinium dioximes over the pyridinium monoximes against dialkylphosphoryl acetylcholinesterase.

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