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Review Article

Oximes Antagonistic to Inhibitors of Cholinesterase

Part II

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PHARMACEUTIC ASPECTS

Experiments with 2-PAM indicated that the iodide form of the oxime would be of little practical value because of its comparatively low solubility in water. The iodide is approximately 2% soluble in water at room temperature (144), while saturated aqueous solutions at 25° contain only 4.8%. In view of the fact that 2-PAM is effective in humans in doses of 20 or more mg./Kg., a volume of at least 30 ml. would be needed for a single injection. Such volumes are obviously impractical for intramuscular injection. There is an additional inherent disadvantage in the use of the iodide form. Large doses of 2-PAM can, and have, elicited symptoms of iodism (145).

In attempting to find more soluble salts of 2-formyl *N*-methyl pyridinium oxime (2FMPO), the nitrate was made by simply adding silver nitrate to 2-PAM (146). Fundamental principles of general chemistry tell us that this reaction should go to completion readily, with the formation of insoluble silver iodide and the nitrate of the oxime. The resulting nitrate was found to be 15 times more soluble than the iodide. Other salts were subsequently synthesized. Table III shows the water solubilities and the percentages of oxime in each salt. The chloride salt (2-PAMCl) on the basis of its excellent water solubility, its high oxime content per mole of compound and, most important, its physiological compatibility, was proposed as the oxime of choice. The methanesulfonate of 2-formyl *N*-methyl pyridinium oxime (2FMPOMS), has been championed by another group of investigators (147). The latter contains 56.6% oxime per mole of compound and is about as soluble as the chloride in water. Equivalent concentrations of various oxime salts reactivate inhibited eel cholinesterase at approximately the same rate; that is, there is little or no effect due to the anion moiety.

Aqueous solutions of the chloride can be autoclaved without significant breakdown, provided that a suitable pH is maintained. Unbuffered 2.5, 10, and 20% solutions of 2-PAMCl showed less than a 4% breakdown when autoclaved at 120° (15 pounds pressure) for 15 minutes. The decomposition of the oxime occurs *via* two pH-

TABLE III.—SOLUBILITY AND PER CENT OXIME MOIETY IN METHYL PYRIDINIUM ALDOXIME SALTS (145)

2-PAM Salt	Solubility, mg./ml. of Soln. at 25°	Percentage Oxime
Chloride	640	79.5
Nitrate	675	68.9
Dihydrogen phosphate	46	58.6
Hydrogen sulfate	640	58.6
Iodide	48	51.9
Fumarate	389	70.6
Acetate	...	69.9
Tartrate	565	64.9
Lactate	1000	60.6

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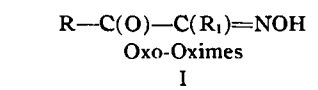
dependent mechanisms. At pH values below 4, the hydrogen ion catalyzes hydrolysis of the acid form of the oxime; various states of equilibrium between 2-PAM and its hydrolytic products, pyridine-2-carboxaldehyde methiodide and hydroxylamine, are established in accord with the pH and temperature of the reaction mixture. Above pH 4, the decomposition is due either to hydroxyl ion catalysis of breakdown of the acid form of the oxime or to noncatalytic, direct attack on the oximate ion. Hydroxyl ion attack at the methine hydrogen results in the removal of a proton and the formation of a carbanion as the rate-controlling step (134). Subsequent loss of hydroxide ion from the oximino nitrogen results in the formation of a triple bond—in this case the corresponding nitrile The nitrile, depending on whether there is direct hydroxide ion attack on the cyano grouping or addition to the pyridine ring, forms carbamidopyridinium or hydroxypyridinium ions. On further reaction with hydroxide ions, the latter forms a pseudo base which loses water to form *N*-methyl-α-pyridone. Ellin (123) showed the presence of 2-cyanopyridinium ion, cyanide ion, and *N*-methyl-pyridone in alkaline hydrolysates of 2-PAM. Kosower and Patton (148) conclusively showed that 2-carbamidopyridine methiodide formed when 2-cyanopyridine methiodide was placed in alkaline media. One would then expect the formation of this amide when 2-PAM is degraded in basic solution; its presence among the products of alkaline degradation of 2-PAM has been confirmed by paper chromatographic techniques.

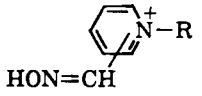
Because cyanide had been found to be a decomposition product of 2-PAM (101, 133), experiments were run to check for the presence of cyanide in aqueous solutions stored over long periods of time. Accelerated storage-stability studies established that the toxicity resulting from 10 and 20% concentrations of oxime was due only to the initial oxime concentration (145). From the established equations for the degradation of PAM, the stability of the oxime in aqueous solution at any pH and temperature may be predicted. Kinetic data show that a solution of 2-PAM, maintained at pH 4.36 at 25°, should retain half of its original concentration of oxime even after a period of 80 years.

Other oximes, such as the aforementioned TMB-4, show promise as antagonists of the toxic effects of organophosphorus compounds, but have not been investigated so extensively as 2-PAM. Experiments concerned with the stability of TMB-4 are complete and are being prepared for publication (149).

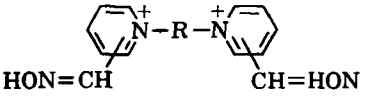
Although chemicals containing the oxime group have been found to possess activity as antibacterial, antifungal, antirickettsial, trypanocidal, tuberculostatic, anticonvulsant, centrally depressant, antispastic, insecticidal, or locally anesthetic agents, probably the most striking biological activity of this type of compound is the ability of some oximes to serve as reactivators of cholinesterase inhibited by certain organophosphorus and carbamate compounds and as antagonists of poisoning by these inhibitors of cholinesterases. The oximes that have been found to have value in these ways fall in general into one of three groups, all mentioned previously:



Oxo-Oximes
I



Monoquaternary Pyridine Aldoximes
II



Bisquaternary Pyridine Bisoximes
III

A few oximes antagonistic to organophosphorus compounds but having other ring structures, including one having the oxime group attached directly to a ring carbon, have been described. These compounds have not exceeded in activity the usual types of oximes.

TOXICITY OF OXIMES

The best known members of the oxo-oxime group (I) are MINA, DAM, and DINA; the best known of the group of monoquaternary pyridine aldoximes (II) are the chloride, iodide, and methanesulfonate salts of 2-formyl *N*-methylpyridinium oxime. Among the bisquaternary pyridine aldoximes (III), the best known is 1,3-bis-(4-formylpyridinium) propane bisoxime dibromide (B4FPBOBr$_2$ or TMB-4). Table IV gives toxicity information about a representative group of oximes. It is apparent that DINA is the most lethal of the group. O'Leary *et al.* (158) have examined the lethal effectiveness in the mouse of i.v. injection of mixtures of equal parts of pyridinium monoximes and bispyridinium bisoximes; in four cases (2FMPOI + B4FPBOBr$_2$, 2FMPOL + B4FPBOBr$_2$, 2FMPOCl + B4FPBOCl$_2$, and 2FMPOMS + B4FPBOBr$_2$), the toxicity of the mixture of

TABLE IV.—TOXICITIES OF SELECTED OXIMES

Oxime[a]	Species	Route	LD_{50}	Ref.
Oximinoacetamide	Mouse	i.p.	4200 mg./Kg.	(100)
MINA	Mouse	i.p.	150	(100)
	Rat (M)	i.p.	50	(150)
	Rat (F)	i.p.	74	(150)
DINA	Mouse	i.p.	20	(100)
DAM	Mouse	i.p.	51, 85, 900	(100, 151, 152)
2-Oximino-3-pentanone	Mouse	i.p.	350	(100)
2FMPOI(2-PAM)	Mouse	i.v.	140–178	(157, 158)
	Mouse	i.p.	136–260	(100, 150–154, 156, 159)
	Mouse	s.c.	290–340	(153, 159)
	Mouse	p.o.	1500–4000	(156, 160)
	Rat	i.p.	305	(150)
2FMPOCl	Mouse	i.v.	115	(158)
	Mouse	i.p.	205	(156)
	Mouse	p.o.	4100	(156)
	Rabbit	i.v.	95	(158)
2FMPOL	Mouse	i.v.	121	(158)
2FMPOMS(P2S)	Mouse	i.v.	118–122	(150)
	Mouse	i.p.	216	(150)
	Mouse	p.o.	3700	(161)
	Rat	i.v.	109	(150)
	Rat	i.p.	262	(150)
2FMPOMS(P2S)	Guinea pig	i.m.	305	(150)
	Rabbit	i.v.	147	(150)
	Monkey	i.m.	356	(150)
$4FPPOBr_2$	Mouse	i.p.	202	(154)
$B4FPBOBr_2$(TMB-4)	Mouse	i.v.	53–89	(157, 158)
	Mouse	i.p.	130	(154)
$B4FPBOCl_2$	Mouse	i.v.	57	(158)
	Rabbit	i.v.	44	(158)

[a] **2FMPOI** = 2-formyl *N*-methylpyridinium oxime iodide (2-PAM); **2FMPOCl** = 2-formyl *N*-methylpyridinium oxime chloride; **2FMPOL** = 2-formyl *N*-methylpyridinium oxime lactate; **2FMPOMS** = 2-formyl *N*-methylpyridinium oxime methanesulfonate; **4FPPOBr$_2$** = 1-(4-formylpyridinium)-3-pyridinium propane oxime dibromide; **B4FPBOBr$_2$** = 1,3-bis(4-formylpyridinium)-propane bisoxime dibromide (TMB-4); **B4FPBOCl$_2$** = 1,3-bis(4-formylpyridinium)-propane bisoxime dichloride.

oximes was between those of the two components and close to the value calculated from the toxicities of the individual components. This last finding suggests that there is no potentiation of the toxicity of $B4FPBOBr_2$ (TMB-4) by either the iodide, the lactate, or the methanesulfonate of 2-formyl *N*-methylpyridinium oxime and none of that of $B4FPBOCl_2$ by the chloride of the monoxime.

EFFECTIVENESS OF OXIMES

Table V summarizes available information about the effectiveness of the same oximes in antagonizing the toxic effects of organophosphorus and carbamate inhibitors of cholinesterases. This table shows several things: (*a*) the oxime with which the greatest volume of work has been done is 2FMPOI (or 2-PAM), (*b*) the only poisoning of humans in which an oxime has been used fairly extensively is that by parathion, (*c*) pyridinium oximes (either mono or bis oximes) are more active than the oxo-oximes, and (*d*) although the oximes are effective antagonists to many of the inhibitors of cholinesterase, there are some anticholinesterase compounds with effects that either are not antagonized by oximes or are made worse by administration of an oxime. In the latter group, Sevin and Diazinon seem to be particularly likely to have their toxic effects enhanced by administration of oximes. This enhancement of toxicity probably occurs through the formation of stable phosphorylated (60, 231) or carbamylated derivatives of the oximes.

The only recourse available today as an aid to atropine in the treatment of severe poisonings by such compounds as Sevin and Diazinon is artificial ventilation. In carrying out this form of therapy, a first requirement is that the airway be rendered patent by removal of secretions and other occlusive material from the pharynx. The maintenance of a patent airway is aided by tilting backwards the head of the supine patient. With a patent airway, one must next insure that the method of artificial respiration being used actually produces effective pulmonary ventilation.

Some of the papers from which the data in Table V are derived contain other information of considerable importance for most effective employment of the oximes in therapy of poisonings by inhibitors of cholinesterase. One such piece of information is that administration of atropine along with the oxime increases the antagonistic activity of MINA against sarin and tabun

TABLE V.—ACTIVITIES OF OXIMES AND SALTS OF OXIMES AS ANTAGONISTS OF LETHAL AND OTHER EFFECTS OF ANTICHOLINESTERASE COMPOUNDS

Oxime	Anti-ChE Compound[a]	Test Object[b]	Antagonism[c]	Ref.
Oximinoacetamide	Sarin	m, r	+	(100)
MINA	Sarin	m, r, gp	+	(59, 100, 102, 120, 144, 163–167)
MINA	Sarin	direct reaction	+	(120, 167)
MINA	Tabun	r	+	(168)
MINA	DFP	r, c	+	(59, 163, 166, 169)
MINA	DFP	rb eye	+	(167, 173, 174)
MINA	TEPP	m; r	−; +	(175; 59, 163, 166)
MINA	DMNP	r	+	(166)
MINA	OMPA; Parathion	m	−; +	(175)
MINA	CH_3-Parathion	m	0	(175)
MINA	Diazinon	m	−	(175)
MINA	EPN, Malathion	m	0	(175)
MINA	Dipterex	m	±	(175)
MINA	Physostigmine	rb eye	+	(171)
DINA	Sarin	m; r	±; +	(100; 59, 163)
DINA	DFP, TEPP	r	+	(59, 163)
DAM	Sarin	m, r, gp, rb, mk; man	+; ±	(59, 100, 102, 120, 144, 158, 164, 165, 171, 172; 218–220)
DAM	Sarin	direct reaction	+	(120)
DAM	Tabun	r	+	(168)
DAM	DFP	r, c	+	(59, 165, 169)
DAM	DFP	rb or human eye	+	(170, 174)
DAM	TEPP	m, r	+	(59, 118, 127, 165, 175)
DAM	OMPA	m, man	±	(175, 218–220)
DAM	Paraoxon; Parathion	m	0	(118; 175)
DAM	CH_3-Parathion	m	−	(175)
DAM	Diazinon; EPN	m	−; ±	(175)
DAM	Malathion; Dimefox	m	−; 0	(175; 118)
DAM	Dipterex	m	±	(175)
DAM	Physostigmine	human eye	+	(170)
DAM	Neostigmine	c; man	+; ±	(123; 218–220)
DAM	Bisneostigmine	man	+	(218–220)
DAM	Bispyridostigmine	man	±	(218–220)
DAM	Ambenonium	man	+	(218–220)
2-Oximino-3-pentanone	Sarin	m, r	+	(100)
2-Oximino-3-pentanone	DFP	rb eye	+	(174)
2FMPOI	Sarin	m, r	±	(100)
2FMPOI	Sarin	m, r, rb, c, d, man	+	(59, 102, 120, 124, 125, 144, 152, 158, 163, 176–181, 182, 218–220)
2FMPOI	Sarin	direct reaction	±	(60, 120)
2FMPOI	Soman	m, rb	0	(181, 182)
2FMPOI	Tabun	m, r, c, d	+	(124, 176)
2FMPOI	DFP	m, r, gp, c, man	+	(59, 81, 82, 116, 117, 154, 155, 163, 178, 183–185)
2FMPOI	DFP	m, rb or human eye	+	(116, 168)
2FMPOI	DFP	direct reaction	+	(185)
2FMPOI	TEPP	m, r, c	+	(59, 117, 123, 127, 154, 160, 163, 175, 176, 187)
2FMPOI	TEPP	direct reaction	+	(186)
2FMPOI	OMPA	m	0; +	(81, 82, 125; 155, 175)
2FMPOI	OMPA	man	±	(218–220)
2FMPOI	Paraoxon	m, r, gp, rb, c	+	(81, 82, 116–118, 121, 122, 151, 155, 178, 184, 188, 189)
2FMPOI	Paraoxon	m eye	+	(116)
2FMPOI	Paraoxon	direct reaction	+	(186)
2FMPOI	Parathion	r	0	(176, 188)
2FMPOI	Parathion	m, r, gp, rb, c, d, h, man	+	(122, 129–132, 151, 153, 175, 176, 178, 184, 189–209)
2FMPOI	CH_3-Parathion	m, r	+	(175, 191, 193, 210)
2FMPOI	Demeton	m, r	+	(191, 211, 213)
2FMPOI	CH_3-Demeton	m, rb	+	(190, 191)
2FMPOI	Isosystox	r	+	(161)
2FMPOI	Phenkaptone	m, r	+	(191, 193)
2FMPOI	CH_3-Phenkaptone	m	0	(191)
2FMPOI	Diazinon	m	−; +	(175; 191)
2FMPOI	Diazinon	r; ct	±; 0	(193; 212)

Oxime	Anti-CHE Compound[a]	Test Object[b]	Antagonism[c]	Ref.
2FMPOI	CH_3-Diazinon	m	0	(191)
2FMPOI	Guthion	r	−	(211)
2FMPOI	C_2H_5-Guthion	r	−	(211)
2FMPOI	EPN	m, man	+	(175)
2FMPOI	Malathion	m; ct; man	−; +; ±	(175; 212; 198)
2FMPOI	Morphothion; Dimethoate	r	±; 0	(211; 193, 211)
2FMPOI	DBD; Thimet	m; r	+	(160; 211)
2FMPOI	Endothion	m, rb	+	(190)
2FMPOI	D600	direct reaction	+	(186)
2FMPOI	I, II, III	m	+	(214)
2FMPOI	Phospholine	m, rb, c	+	(170, 216)
2FMPOI	Phospholine	rb or human eye	+	(170, 215)
2FMPOI	Phosdrin	r, man	+	(211, 217)
2FMPOI	$R_0$3-0340, $R_0$3-0351, $R_0$3-0422	m	+	(117, 154)
2FMPOI	$R_0$3-0340, $R_0$3-0422	direct reaction	+	(186)
2FMPOI	Dimefox	m; r	+; 0	(118; 193, 211)
2FMPOI	Phosphamidon	m, r	+	(211, 221, 222)
2FMPOI	Dipterex	m	+	(175)
2FMPOI	Physostigmine	gp	+	(184)
2FMPOI	Physostigmine	rb or human eye	+	(170)
2FMPOI	Neostigmine	m; man	0; +	(154, 217; 218–220)
2FMPOI	Bisneostigmine	man	+	(218–220)
2FMPOI	Mestinon	m	±	(217)
2FMPOI	Bispyridostigmine	man	±	(218–220)
2FMPOI	Dimetilan	m; r	+; ±	(191, 223; 211)
2FMPOI	Isolan	m; r	±; +	(223; 211)
2FMPOI	Sevin	r	−	(204, 211)
2FMPOI	Humorsol	m	±	(217)
2FMPOI	Pyramat; Pyrolan	m	+; ±	(223)
2FMPOI	G23091; Dimetan	m	+; 0	(223)
2FMPOI	Ambenonium	m; man	0; +	(216; 218–220)
2FMPOI	Edrophonium	m	0	(216)
2FMPOCl	Sarin, Tabun	rb	+	(158)
2FMPOL	Sarin	rb, d	+	(158, 225)
2FMPOMS	Sarin	m, r, gp, rb, d	+	(150, 158, 161, 164, 225, 226)
2FMPOMS	Tabun	m, r, gp, rb	+	(158, 161, 164)
2FMPOMS	DFP	m, r, gp, rb	+	(161)
2FMPOMS	DFP	rb eye	+	(174)
2FMPOMS	TEPP	m, r, gp, rb	+	(150, 161)
2FMPOMS	OMPA	m, r, gp, rb	+	(161)
2FMPOMS	Paraoxon	m, r, gp, rb	+	(161)
2FMPOMS	Demeton	r	+	(211)
2FMPOMS	Isosystox	m, r, gp, rb	+	(161)
2FMPOMS	Vinylphos	m, r, gp, rb	+	(161)
2FMPOMS	Guthion, C_2H_5-Guthion	r	+	(211)
2FMPOMS	Morphothion; Thimet	r	0; +	(211)
2FMPOMS	Phospholine	rb eye	+	(174)
2FMPOMS	Amiton	m, r, gp, rb	+	(161, 226)
2FMPOMS	S-l, Ch-l	r	+	(225)
2FMPOMS	Phosdrin, Phosphamidon	r	+	(211)
2FMPOMS	Physostigmine	m, r, gp, rb	+	(162)
2FMPOMS	Dimetilan	r	±	(211)
2FMPOMS	Isolan; Sevin	r	+; −	(211)
2FMPOMS	Humorsol	rb eye	+	(174)
4FPPOBr$_2$	Sarin, DFP	m	+	(154, 187)
4FPPOBr$_2$	TEPP, $R_0$3-0340	m	+	(154, 187)
B4FPBOBr$_2$	Sarin	m, r, rb, c, d	+	(89, 158, 164, 172, 177, 182, 187, 228)
B4FPBOBr$_2$	Soman	m, r	±	(182)
B4FPBOBr$_2$	Tabun; DFP	r; m	+	(168; 154, 172, 228)
B4FPBOBr$_2$	DFP	rb or human eye	+	(170)
B4FPBOBr$_2$	TEPP; OMPA	m	+; 0	(86, 127, 154, 187; 229)
B4FPBOBr$_2$	Paraoxon	m	+	(214)
B4FPBOBr$_2$	Parathion, CH_3-Parathion	r	+	(193)
B4FPBOBr$_2$	Armin, Phenkaptone	m, r	+	(193, 229)
B4FPBOBr$_2$	Diazinon; Dimethoate	r	±; 0	(193)
B4FPBOBr$_2$	I, II, III	m	+	(214)
B4FPBOBr$_2$	Phospholine, $R_0$3-0340	m	+	(154, 215)
B4FPBOBr$_2$	Dimefox; Phosphamidon	m	0; +	(193, 229; 221, 229)
B4FPBOBr$_2$	Dipterex	m	+	(229)
B4FPBOBr$_2$	Physostigmine	rb or human eye	+	(170)
B4FPBOBr$_2$	Neostigmine; Bisneostigmine	m	+; 0	(154, 229; 154)

Oxime	Anti-ChE Compound[a]	Test Object[b]	Antagonism[c]	Ref.
$B4FPBOBr_2$	Mestinon	m	0; +	(229; 216)
$B4FPBOBr_2$	Bispyridostigmine, Isolan	m	0	(154, 216, 229)
$B4FPBOBr_2$	Humorsol	m	0; ±	(229; 216)
$B4FPBOBr_2$	Dimetan; Ambenonium	m	0; −	(229; 216)
$B4FPBOBr_2$	Edrophonium	m	0	(216)
$B4FPBOCl_2$	Sarin; Soman	rb; c	+; ±	(158, 227; 230)
$B4FPBOCl_2$	Tabun	rb	+	(158)

[a] Symbols are: **I**: Diethyl-*p*-nitrophenyl phosphonate; **II**: Methyl-*O*-ethyl-*p*-nitrophenyl phosphonate; **III**: Methyl-*O*-isopropyl-*p*-nitrophenyl phosphonate; **Amiton**: *O,O*-Diethyl-S-(2-diethylaminoethyl) phosphonothioate; **Armin**: Ethyl-4-nitrophenyl ethylphosphonate; **Ch−1**: Isopropyl methyl phosphonothiocholinate; **D-600**: Diisopropyl-4-nitrophenylphosphate; **DBD**: *O,O*-Dimethyl-S-(4-oxo-3-H-1,2,3-benzotriazine-3-methyl) phosphorodithioate; **Demeton**: *O,O*-Diethyl-*O*-(2-ethylthioethyl) phosphorothioate; **DFP**: Diisopropyl phosphorofluoridate; **Diazinon**: *O,O*-Diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate; **Dimefox**: Tetramethyl phosphorodiamidic fluoride; **Dimetan**: 5,5-Dimethyldihydroresorcinol dimethylcarbamate; **Dimethoate**: *O,O*-Dimethyl-S-(*N*-methyl-carbamylmethyl) phosphorodithioate; **Dimetilan**: 2-Dimethylcarbamyl-3-methylpyrazolyl-5-dimethylcarbamate; **Dipterex**: Dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate; **DMNP**: Dimethyl-4-nitrophenylphosphate; **Edrophonium**: *N*-Dimethyl-*N*-ethyl-*m*-hydroxyanilinium bromide; **Endothion**: 2-(Dimethoxyphosphinylthiomethyl)-5-methoxy-4-pyrone; **EPN**: *O*-Ethyl-*O*-(4-nitrophenyl) phenylphosphonothioate; **Ethylgluthion**: *O,O*-Diethyl-S-(4-oxo-3-H,1,2,3-benzotriazine-3-methyl) phosphorodithioate; **G23091**: 2,6-Dimethyl pyridyl-4-dimethylcarbamate; **Guthion**: *O,O*-Dimethyl-S-(4-oxo-3-H,1,2,3-benzotriazine-3-methyl) phosphorodithioate; **Humorsol**: Decamethylene bis [*N*-methylcarbamic acid *m*-dimethylaminophenyl ester] bromoethylate; **Isolan**: 1-Isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate; **Iso-Systox**: *O,O*-Diethyl-S-(2-ethylthioethyl)phosphorothioate; **Malathion**: *O,O*-Dimethyl-S-(1,2-dicarbethoxyethyl) thiothionophosphate; **Mestinon**: 1-Methyl-3-hydroxypyridinium bromide dimethylcarbamate; **Methyldemeton**: *O,O*-Dimethyl-*O*-(2-ethylthioethyl) phosphorothioate; **Methyldiazinon**: *O,O*-Dimethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothioate; **Methylparathion**: *O,O*-Dimethyl-*O*-(4-nitrophenyl) phosphorothioate; **Methylphenkaptone**: *O,O*-Dimethyl-S-(methylthio-2,5-dichlorophenyl) phosphorodithioate; **Morphothion**: *O,O*-Dimethyl-S-(*N*-morpholino carbamylmethyl) phosphorodithioate; **OMPA**: Tetramonoisopropyl pyrophosphortetramide; **Paraoxon**: Diethyl-4-nitrophenylphosphate; **Parathion**: *O,O*-Diethyl-*O*-(4-nitrophenyl) phosphorothioate; **Phenkaptone**: *O,O*-Diethyl-S-(methylthio-2,5-dichlorophenyl) phosphorodithioate; **Phosdrin**: Dimethyl-1-methyl-2-carbomethoxyvinyl phosphate; **Phosphamidon**: Dimethyl-1-methyl-2-chloro-2-*N,N*-diethylcarbamylvinyl phosphate; **Phospholine**: *O,O*-Diethyl-S-2-trimethylammonium-ethyl phosphonothiolate iodide; **Pyramat**: 2-Propyl-4-methylpyrimdyl-6-dimethylcarbamate; **Pyrolan**: 3-Methyl-1-phenyl-5-pyrazolyl dimethylcarbamate; **Ro3-0340**: 3-(Diethylphosphato)-*N*-trimethylanilinium-methyl sulfate; **Ro3-0351**: 3-(Diisopropylphosphato) pyridine; **Ro3-0422**: 3-(Diethylphosphato)-*N*-methyl-quinolinum methyl sulfate; **S-1**: Isopropylmethyl-S-dimethylaminoethyl phosphonothiolate; **Sarin**: Isopropyl methylphosphonofluoridate; **Sevin**: 1-Naphthyl-*N*-methylcarbamate; **Soman**: Pinacolyl-methylphosphonofluoridate; **Tabun**: Ethyl-*N*-dimethyl phosphoramido cyanidate; **TEPP**: Tetraethyl pyrophosphate; **Thimet**: *O,O*-Diethyl-S-ethylthio-methyl phosphorodithioate; **Vinylphos**: Diethyl-2-chlorovinylphosphate.

[b] Symbols are: m = mouse, r = rat, gp = guinea pig, rb = rabbit, c = cat, d = dog, mk = monkey, h = horse, and ct = cattle.

[c] Symbols are: − = administration of oxime increases severity of toxic or other effect of the anti-ChE compound; 0 = administration of oxime has no effect on the toxicity or other action of the anti-ChE compound; ± = administration of oxime may decrease toxicity or other effect of the anti-ChE compound; + = administration of oxime decreases toxicity or other action of the anti-ChE compound. The semicolon is used to separate entries including disparate or qualitatively different effects.

(167, 168), of DAM against sarin and tabun (144, 164, 168), of 2FMPOI or 2FMPOMS against sarin, tabun, DFP, TEPP, OMPA, paraoxon, parathion (−176, +190). CH_3-Demeton, endothion, S-1, CH-1, Ro3-0340, Ro3-0351, Ro3-0422 and phosphamidon (89, 116, 117, 124, 154, 164, 176, 177, 183, 184, 190, 222, 225), of $4FPPOBr_2$ against DFP and TEPP (154), and of $B4FPBOBr_2$ against sarin, tabun, DFP, TEPP and Ro3-0340 (89, 154, 164, 168, 177). On the other hand, the mixture of atropine and oxime has no significant advantage against guthion, C_2H_5-guthion, Demeton, and morphothion (211). Still, it appears that, as a general rule, the oxime should be used along with atropine sulfate. The necessity for large doses of atropine in treating poisoning by cholinesterase inhibitors has been pointed out many times (130, 131, 196, 218, 232–235).

The dosages for the only oximes used so far to treat intoxication by inhibitors of cholinesterase in man, DAM and 2FMPOI appear to be quite similar (218). Grob and Johns (218) recommend total intravenous doses of 2000 mg. of either oxime, given at rates not greater than 500 mg./minute for 2FMPOI or 200 mg./minute for DAM, in severe intoxication with cholinesterase inhibitors. This dose may be repeated if muscular weakness is not relieved or recurs. In less severe intoxication, total doses of 1000 mg. of either oxime may be used similarly. Durham and Hayes (236) recommend total intravenous doses of 1000 mg. of 2FMPOI in either severe or moderate poisoning by anticholinesterase compounds. Other salts of 2FMPO may be used similarly.

The finding of Crook *et al.* (224) that salts of 2FMPO administered orally prior to exposure to lethal concentrations of sarin vapor are effective in protecting dogs subsequently exposed to sarin and then treated by intramuscular injection of 5 mg./Kg. of atropine sulfate, suggests that prophylactic employment of such salts may be useful in cases of possible exposures to cholinesterase inhibitors. This possibility has not been reduced to practice, however.

Certain of the references in Table V compare $B4FPBOBr_2$ with 2FMPOI and conclude that the former compound is the more active and the more rapidly effective against poisoning by sarin, tabun, TEPP, paraoxon, I, II, III, and phosphamidon (86, 127, 154, 168, 177, 187, 214, 221, 228). Despite these apparently advantageous properties, no salt of B4FPBO has been used to treat intoxications of men by inhibitors of cholin-

esterase. The lack of interest in the bis-quaternary type of oxime for therapy may be due to the increased lethality of this type of oxime (Table IV) as well as to the toxic actions of the compound.

The principal toxic effects of B4FPBO are related to its being a bis-quaternary compound: they are a curariform block of neuromuscular transmission and a hexamethonium-like depression of blood pressure (237). This oxime recently has been found to be irritating to the human stomach when daily oral doses were given (238). 2FMPOMS has been reported (239) to produce fibrosis of the mucous membrane of the stomach in dogs given the material by mouth on 5 days a week for 17 weeks. The most striking toxic effects of 2FMPOI are (*a*) the production of pharyngeal pain and enlargement of the parotid glands in some men—effects that seem to be related to the iodide ion and that are absent from 2FMPOCl and 2FMPOMS—and (*b*) the production by large doses of block of neuromuscular transmission (123, 163, 218). Other toxic effects of DAM and 2FMPOI in man have been reported by Jager and Stagg (240). Daily doses of as much as 6 Gm. of 2FMPOCl have been given by mouth to humans for many weeks without signs of gastric discomfort or of any other sort of toxicity (238).

Salts of 2FMPO increase the pressor response to injections of catecholamines (238, 241). This pressor-sensitizing effect of salts of 2FMPO enables single doses of mixtures of these compounds and of salts of B4FPBO to be administered parenterally to experimental animals without marked deleterious effect on blood pressure and with excellent therapeutic activity against poisonings by sarin or tabun (158).

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Research Articles

Spectrophotometric Effect of Salicylic Acid and Selected Antiradiation Agents on Catalase and Lactic Dehydrogenase

By WILLIAM O. FOYE and PHILIP S. THIBODEAU*

Spectrophotometric measurements have been made of systems of catalase and of lactic dehydrogenase containing either salicylic acid, 2-mercaptoethylamine, 2-mercaptoethylguanidine, or sodium diethyldithiocarbamate. Absorption differences indicated complex formation between the dithiocarbamate and lactic dehydrogenase and at least salt formation with catalase. No definite indication of complex formation between these enzymes and either salicylic acid or the basic mercaptans was given by the method employed, although salt or weak field complex formation is a possibility with the latter.

COMPLEXATION of the metal constituents of enzymes by drugs or biologically active molecules has been postulated as a possible mechanism of drug action. Enzyme inhibition by such molecules is often believed to involve complexation of the active catalytic site, which is frequently the metal. Little definite evidence has been provided to demonstrate such complex formation, however, although Vallee has been able to demonstrate enzyme-chelating agent complexations involving the metal constituent by means of both ultraviolet absorption spectral changes (1) and optical rotatory dispersion studies (2). Keilin and Hartree (3) have shown complexation of catalase with such inhibitors as cyanide, sulfide, fluoride, and azide by means of ultraviolet absorption, and Chance (4) has been able to demonstrate the existence of short-lived catalase-peroxide complexes spectrophotometrically using a rapid-flow technique. In these cases, significant differences in either absorption maxima or absorbance of the enzyme were observed.

No less than 30 enzymes are known to be affected by the presence of salicylates *in vivo*, *in vitro*, or both, and many of these enzymes are now recognized as metal-containing. Nitzescu and Cosma (5) reported a powerful depressing action on the succinodehydrogenases by salicylate; von Euler and Ahlstrom (6) stated that salicylate inhibits the activity of lactic dehydrogenase (LDH). Baker (7) later found that salicylate inhibited the conversion of lactate to pyruvate by means of LDH catalysis. Tomimura (8) has described the inhibition of a peroxide-catalase system by salicylate, and

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