TREATMENT OF POISONING BY ANTICHOLINESTERASE INSECTICIDES IN THE RAT

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The effects of an oxime cholinesterase reactivator, 2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate (pralidoxime methanesulphonate, P2S) or iodide (pralidoxime iodide, PAM, P2AM), and atropine, given separately or together, on poisoning by ten organophosphate or carbamate anticholinesterase insecticides are reported on rats. Atropine alone was beneficial with all materials. Oxime therapy alone was effective to varying degrees with isolan, phosdrin, gusathion, ethyl-gusathion, demeton, thimet, phosphamidon and possibly dimetilan, but ineffective with sevin and morphothion. With the organophosphates, these findings are related to the proportion of reversible enzyme inhibition predicted. No potentiation between atropine and oxime was found when the insecticide was given orally, and the combination was less effective than atropine alone with gusathion, ethyl-gusathion, demeton and morphothion. Beneficial potentiation between oxime and atropine did occur with intraperitoneal morphothion.

Previous work by Sanderson and Edson (1959) on oxime therapy for poisoning by organophosphorus insecticides in the rat showed that the relative effectiveness of therapy by oxime cholinesterase reactivators could be related to the proportion of reversible cholinesterase inhibition present, as predicted from the structure and duration of action of the organophosphate. With the slower acting indirect inhibitors of cholinesterase now commonly used as insecticides, repeated doses of oxime are necessary. Oxime injections were beneficial in poisoning by parathion, parathion-methyl, phenkaptone and, to a lesser extent, diazinon, but ineffective with dimefox and dimethoate.

Some studies have been published of oxime therapy for poisoning by other commercial organophosphate insecticides. Work summarised by Davies and Green (1959) showed that oxime therapy is ineffective with schradan poisoning, due to irreversibility of inhibition, though a conflicting report by Wills (1959) does suggest some beneficial effect. Fournel (1957a, b) showed that pralidoxime iodide therapy reduced mortality with endothion, and to a lesser extent with demeton-methyl. Wills (1959) also reports that pralidoxime iodide is effective against EPN and dipterex, but not against malathion or diazinon, while Bergner (1959) reports that it can reactivate tissue cholinesterase after diazinon poisoning. With the exception of the findings of Wills (1959) on schradan and possibly diazinon, these observations are consistent with expectations from predicted proportions of reversible cholinesterase inhibition.

A number of carbamate anticholinesterase insecticides are now available. The only known reports on oxime therapy of poisoning by carbamate insecticides are those by Smyth, Carpenter, Nair, Palm, Rogers,

TABLE I

EFFECT OF IMMEDIATE ATROPINE AND OXIME THERAPY ON RATS GIVEN ORAL INSECTICIDE

Compound	Rat sex	LD50, mg./kg.	Dose, mg./kg.	Therapy	Deaths	Time of onset, min.	Time of death	Remarks
Isolan	M	12	20	Nil	5/6	1	4–29′	
				Atropine	1/6	2	18′	Effective, animals dry.
				P2S	2/6	2	22′	No effect till convulsions, then reduced mortality.
				P2AM	1/6	2	24′	No effect till convulsions, then reduced mortality.
				Atropine/P2S	0/6	2	—	Effects as atropine group.
				Atropine/P2AM	1/6	7	16′	Effects as atropine group.
Sevin	F	400	700	Nil	6/6	6	45′–23 hr.	
				Atropine	1/6	10	25′	Effective, animals dry.
				P2AM	6/6	8	10′–26 hr.	No benefit. Deaths apparently faster than control.
				Atropine/P2AM	2/6	14	22 hr.–3d.	Effects as atropine group.
Dimetilan	F	25	50	Nil	6/6	2	4–33′	
				Atropine	2/6	1	26′	Effective, animals dry.
				P2AM	5/6	2	14–30′	Slight benefit.
				Atropine/P2AM	1/6	5	20′	Effects as atropine group.
Phosdrin	M	5	8	Nil	5/6	5	7–16′	
				Atropine	1/6	11	12′	Effective, animals dry.
				P2S	1/6	2	19′	No effect till convulsions, then reduced mortality.
				P2AM	2/6	5	35′	No effect till convulsions, then reduced mortality.
				Atropine/P2S	0/6	5	—	Effects as atropine group.
				Atropine/P2AM	0/6	6	—	Effects as atropine group.
Gusathion	F	7	12	Nil	4/6	6	22–35′	
				Atropine	0/6	9	—	Effective, animals dry.
				P2AM	0/6	6	—	Effective, symptoms reduced.
				Atropine/P2AM	1/6	14	28′	Effects as atropine group, but recovery slower.

TABLE I—*continued*

Compounds	Rat sex	LD50 mg./kg.	Dose, mg./kg.	Therapy	Deaths	Time of onset, min.	Time of death	Remarks
Ethyl-gusathion	F	9·5	14	Nil	6/6	10	50′–2d.	
				Atropine	0/6	17	—	Effective, animals dry.
				P2AM	3/6	5	50′–20 hr.	Lower mortality, symptoms unaffected.
				Atropine/P2AM	3/6	15	35′–4d.	Effects as atropine group, greater mortality.
Morphothion*	M	200	300	Nil	6/6	50	2½–23 hr.	
				Atropine	2/6	29	2d.	Effective, animals dry.
				P2AM	5/6	12	20′–25 hr.	No benefit.
				Atropine/P2AM	6/6	3	7′–28 hr.	Effects faster than control group.
Demeton*	F	2·7	3·5	Nil	5/6	30	1½–2½ hr.	
				Atropine	0/6	35	—	Effective, animals dry.
				P2AM	1/6	20	24′	Effective, symptoms reduced.
				Atropine/P2AM	3/6	10	20′	Effects more severe than atropine group.
Thimet*	M	3	4	Nil	6/6	36	3–23 hr.	
				Atropine	3/6	40	6 hr.–2d.	Effective, animals dry.
				P2AM	5/6	53	5 hr.–2d.	Deaths slower, symptoms otherwise unaffected.
				Atropine/P2AM	3/6	50	5 hr.–2d.	Effects as atropine group.
Phosphamidon*	F	15	20	Nil	6/6	10	25–35′	
				Atropine	0/6	14	—	Effective, animals dry.
				P2AM	0/6	17	—	Effective, symptoms still severe.
				Atropine/P2AM	0/6	10	—	Effects as atropine group.

* Therapeutic injections repeated subcutaneously after 4 hr.

Weil and Woodside (1958) and by Carpenter, Weil, Palm, Woodside, Nair and Smyth (1961) who found pralidoxime iodide ineffective against sevin poisoning in dogs and rats.

It is generally accepted (Davies and Green, 1959) that where oxime therapy is effective, there is a beneficial potentiating effect between the oxime and atropine, so that the two drugs given together produce greater benefit than either separately. Sanderson and Edson (1959) found indications that supplementing atropine by pralidoxime iodide in rats poisoned by oral dimethoate could reduce the beneficial effect of the atropine and give a higher mortality. Some of the results of Fournel (1957a) on mice poisoned orally by parathion, endothion or demeton-methyl are open to similar interpretation.

It thus seemed desirable to study, on others of the many commercially available anticholinesterase insecticides, the effectiveness of oxime therapy with and without atropine.

Materials and Methods

Of the 10 insecticides tested (whose structures are included in Table III), phosdrin, gusathion, ethyl-gusathion, morphothion, demeton and phosphamidon were commercial liquid formulations, administered undiluted;

TABLE II

Effect of atropine and pralidoxime iodide therapy subcutaneously on male rats given 150 mg./kg. intraperitoneal morphothion

Therapy*	Mortality	Time of onset, min.	Time of death, hr.	Remarks
Nil	2/6	35	4½–22	
Atropine	0/6	30	—	Effective, animals dry
Pralidoxime iodide	3/6	35	3–22	No benefit
Atropine + pralidoxime iodide	0/6	50	—	Animals much less affected than atropine group, and dry

* Given subcutaneously immediately and repeated after 4 hr.

isolan and thimet were liquid technical samples, administered undiluted; sevin and dimetilan were technical samples administered in glycerol formal (Sanderson, 1959). A pure sample of morphothion was also used, in glycerol formal, for intraperitoneal injection. 2-Hydroxyiminomethyl-*N*-methylpyridinium iodide (pyridine-2-aldoxime methiodide, pralidoxime iodide, P2AM, PAM), obtained from Messrs. L. Light and Company and the methanesulphonate (pyridine-2-aldoxime methyl methanesulphonate, pralidoxime methanesulphonate, P2S), obtained from Aldrich Chemical Co. Inc., and B.P. grade atropine sulphate were all administered in aqueous solution.

Rats were semi-adult (150–250 g.) animals of Wistar strain, maintained and fed under standard conditions. Administration techniques were orthodox, and animals were observed for 7 days.

In most experiments, the insecticide was given orally, followed immediately by intraperitoneal injections of the appropriate therapeutic drugs. With the relatively slower acting insecticides morphothion, demeton,

phosphamidon and thimet, further subcutaneous therapeutic injections were given 4 hr. later. Doses of the therapeutic drugs were standardised at 100 mg./kg. for pralidoxime iodide and methane-sulphonate, and 17·4 mg./kg. for atropine sulphate, these doses alone having previously been shown to be non-toxic. Oral insecticide doses were chosen to be just above the previously determined LD50.

Observations were based on appearance of the animals and rate of development of effects, as well as on mortalities. Mortality differences of one animal between groups were ignored, and differences of two, regarded arbitrarily as borderline. The results did not permit statistical examination.

RESULTS

The results of tests on the effects of oximes and atropine on oral poisoning by the ten insecticides in groups of six rats are summarised in Table I. In a further test, summarised in Table II, the effects of subcutaneous therapeutic injections immediately and after 4 hr. on groups of six rats poisoned by intraperitoneal morphothion, pure, in glycerol formal, were examined.

All of the insecticides caused typical anticholinesterase effects, but at different rates. All showed reduced mortality with atropine therapy,

TABLE III

RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND EFFECTIVENESS OF OXIME THERAPY
(Insecticide given orally)

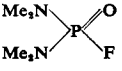
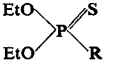
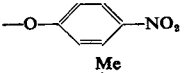
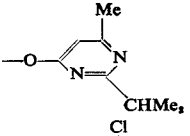
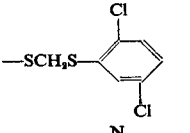
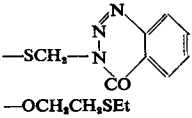
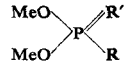
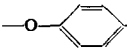
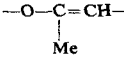
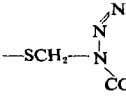
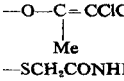
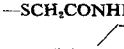
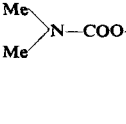
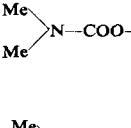
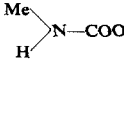
Compound	Structure	Speed of toxic action	Relative benefit from oxime
Dimefox		Slow	None
			
	R		
Parathion		Moderate	Marked
Diazinon		V. slow	Moderate
Phenkaptone		Slow	Marked
Ethyl-gusathion		Moderate	Moderate
Demeton	—OCH_2CH_2SEt	Moderate	Marked
Thimet	—SCH_2—SEt	Slow	Slight

TABLE III—*continued*

Compound	Structure		Speed of toxic action	Relative benefit from oxime
				
	R	R′		
Parathion-methyl		S	Rapid	Marked
Phosdrin		O	Rapid	Marked
Gusathion		S	Rapid	Marked
Phosphamidon		O	Moderate	Marked
Dimethoate		S	Slow	None
Morphothion		S	Slow	None
Isolan			V. rapid	Marked
Dimetilan			V. rapid	Slight
Sevin			Rapid	None

accompanied by reduced symptom intensity and particularly by suppression of salivation, lachrymation and urinary incontinence.

Effects of oxime therapy varied from one compound to another, as shown in Table I. With some compounds symptoms and mortality were both reduced, with some, mortality was reduced or delayed with little effect on symptoms until that stage, with some, there was no apparent effect, while with one, sevin, there was a suggestion of faster deaths.

Atropine and oxime given together never showed a greater benefit than atropine alone after oral insecticide, but did so after intraperitoneal morphothion. With some insecticides the combination was less beneficial than atropine alone, this being most marked with oral morphothion where the combination produced more severe effects than no therapy at all. There was no question of direct toxic effects from the therapeutic injections, these being readily distinguishable and excluded at these doses by

control tests. In the two compounds with which pralidoxime methanesulphonate was tested, no difference was detected between its beneficial effects and those of the same dose of the iodide.

Discussion

As might be expected, atropine therapy was beneficial with all the compounds tested.

Oxime therapy alone was beneficial with the diethyl phosphate insecticides ethyl-gusathion, demeton and thimet, and with the quicker-acting dimethyl phosphates phosdrin, gusathion and phosphamidon, but not with the slow-acting dimethyl phosphate morphothion, either orally or intraperitoneally. These findings are thus in accord with predicted proportions of reversible cholinesterase inhibition, since of these materials only morphothion would be expected to give largely irreversible inhibition. These and previous results (Sanderson and Edson, 1959) are related to chemical structure in Table III.

Of the carbamate insecticides tested, oxime therapy alone was beneficial with isolan, probably slightly with dimetilan, and not with sevin. Of these, sevin is an *N*-monomethyl carbamate, while isolan and dimetilan are *NN*-dimethyl carbamates. The result with sevin agrees with those of Smyth, Carpenter, Nair, Palm, Rogers, Weil and Woodside (1958), and Carpenter, Weil, Palm, Woodside, Nair and Smyth (1961). Effects due to the *NN*-dimethyl carbamate anticholinesterase neostigmine and some of its derivatives were reversed by oxime administration (Grob and Johns, 1958). Insufficient work has yet been done on reactivation of carbamoylated cholinesterase to enable any reasonable theories relating structure and ease of oxime reactivation to be propounded.

It is noteworthy that none of the tests of Table I showed any increased benefit when oxime was given as well as atropine after oral administration of insecticide. Most workers who have noted potentiation between the two therapeutic drugs (Davies and Green, 1959), however, have administered their anticholinesterases by injection. In these tests, potentiation of beneficial effect was, in fact, seen after intraperitoneal morphothion (Table II). In the tests of Table I, it was found that pralidoxime iodide apparently reduced the beneficial effect of atropine with gusathion, ethyl-gusathion, demeton and morphothion given orally, the toxic effects with morphothion being more severe and faster than with no therapy at all. With demeton and ethyl-gusathion, the apparent deleterious effect of the combination occurred where pralidoxime iodide alone was beneficial. Similar apparent deleterious effects when oral anticholinesterase poisoning is treated with oxime and atropine have been noted by Sanderson and Edson (1959) with dimethoate, and possibly unwittingly by Fournel (1957a) with parathion, endothion and demeton-methyl. This finding was at first sight difficult to account for except possibly in terms of an interference by the combined therapeutic drugs with activating or detoxifying enzyme systems, thus indirectly altering the proportion of reversible cholinesterase inhibition. A further possibility, though most unlikely, is that the effect may be partly due to formation of a more toxic

compound between inhibitor and oxime, as has been reported with sarin (Hackley, Steinberg and Lamb, 1959). However, it has recently been suggested (F. Hobbiger, personal communication) that the combination might effect absorption rate of the insecticide from the gut after oral administration, partly by reducing pyloric peristalsis, giving a higher and earlier tissue concentration of inhibitor, and greater persistence also. It is not easy to explain all the effects observed on this basis, but it could well account for the deleterious effect apparently only occurring after poisoning by some compounds given orally, as is suggested by these results.

It is thus apparent that oxime therapy alone is not beneficial with poisoning by all anticholinesterases, and that with organophosphates it is usually possible to predict whether oximes will be beneficial on the basis of expected proportion of reversible cholinesterase inhibition. Even if oxime therapy is not beneficial, it is not harmful alone or, in many cases, in the presence of atropine. It is suggested that any occasional adverse effects of combined therapy might occur only immediately after oral poisoning. If Hobbiger's suggestion of an effect on gut absorption is correct, any adverse effect of giving oxime with atropine should be reduced if absorption of the poison from the gut is largely complete before therapy is commenced, and absent after poisoning by skin absorption. Some further exploration of factors affecting the combined effects of oxime and atropine therapy for anticholinesterase poisoning under different conditions seems desirable.

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