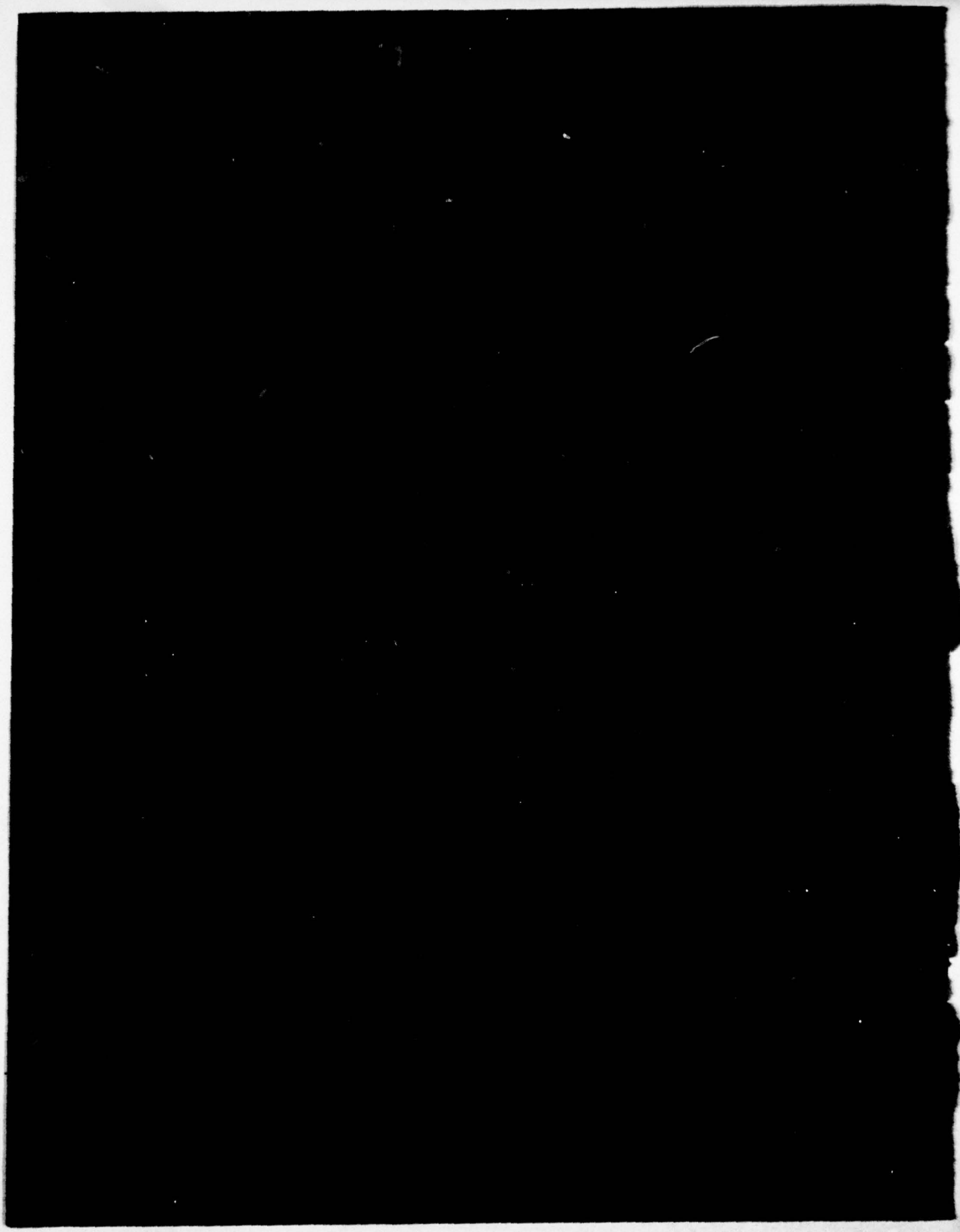
AD-A058 683 FEDERAL AVIATION ADMINISTRATION WASHINGTON D C OFFICE--ETC F/G 6/15
REACTIONS OF METHAMIDOPHOS WITH MAMMALIAN CHOLINESTERASE,(U)
JUL 78 C P ROBINSON, D BEIERGROHSLEIN

UNCLASSIFIED FAA-AM-78-26 NL

1 OF 1
AD A058 683

END
DATE FILMED
11-78
DDC



Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
FAA-AM-78-26		

4. Title and Subtitle	5. Report Date
Reactions of Methamidophos with Mammalian Cholinesterases	July 1978
	6. Performing Organization Code
7. Author(s) Casey P. Robinson,** Donald Beiergrohslein, ** Paul W. Smith,* and Charles R. Crane*	8. Performing Organization Report No.
9. Performing Organization Name and Address * FAA Civil Aeromedical Institute P.O. Box 25082 Oklahoma City, Oklahoma 73125 ** College of Pharmacy, O.U., HSC, Oklahoma City, OK 73190	10. Work Unit No. (TRAIS)
	11. Contract or Grant No.
12. Sponsoring Agency Name and Address Office of Aviation Medicine Federal Aviation Administration 800 Independence Avenue, S.W. Washington, D.C. 20591	13. Type of Report and Period Covered
	14. Sponsoring Agency Code FAA

15. Supplementary Notes

Research leading to preparation of this report was performed under task AM-A-75-TOX-28.

16. Abstract

The lethality of methamidophos, a phosphoramidothioate, to rats (i.p. LD_{50}, 15 mg/kg), is similar to that of such potent organophosphate compounds as parathion and paraoxon. Certain distinctive features of its chemical structure, and reported failure of cholinesterase inhibited with methamidophos to reactivate spontaneously in insects, prompted this study of its reactions with mammalian cholinesterase to determine if the treatment of poisoning requires modification. Atropine (10 mg/kg) or pralidoxime (60 mg/kg) afforded significant protection against lethality from methamidophos (LD_{50}'s, ~~60~~ $\pm$ 0.4 and 52 $\pm$ 4.9 mg/kg, respectively). Partial spontaneous recovery of inhibited cholinesterase activity was observed. However, a single dose of pralidoxime, given essentially simultaneously with methamidophos, did not hasten the recovery of cholinesterase activity.

17. Key Words	18. Distribution Statement
Methamidophos Cholinesterase inhibition Cholinesterase reactivation Methamidophos toxicity Organophosphorus insecticides	Document is available to the public through the National Technical Information Service, Springfield, Virginia 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	7	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

REACTIONS OF METHAMIDOPHOS WITH MAMMALIAN CHOLINESTERASE

I. Introduction.

Methamidophos (O-methyl, S-methyl phosphoramidothioate, Monitor) is an effective insecticide-acaricide, comparable in mammalian toxicity to such older and more familiar organophosphorus cholinesterase inhibitors as parathion and methylparathion. Certain distinctive features of its chemical structure and of its reported toxic mechanisms (Quistad et al., 1970) in insects prompted us to take an in-depth look at the dynamics of its reactions with mammalian cholinesterase (ChE).

Methamidophos is highly toxic to the common housefly, Musca domestica L., exhibiting a topical LD_{50} of 1.3 µg per gram (Quistad et al., 1970). Its insecticidal effectiveness against this species appears to result from a favorable balance between two properties which tend to oppose each other. It does not react rapidly with housefly-head ChE. However, the enzyme-inhibitor complex, once formed, does not dissociate and there is no spontaneous reactivation of the enzyme (Quistad et al., 1970).

These characteristics are in sharp contrast with the reaction of methylparaoxon, the active derivative of methylparathion, on mammalian ChE. Methylparaoxon reacts rapidly with the enzyme but allows significant spontaneous reactivation before the "aging" process, common to all organophosphates, can destroy the enzyme. In view of these differences, it is worthy of note that methylparaoxon is not much more toxic to higher animals than is methamidophos.

Thus, it becomes important to know how methamidophos reacts with mammalian ChE, because it is on such information that rational therapy of acute poisoning must be based, should such occur among aerial applicator personnel or others who handle this pesticide.

In the present investigation, the reactions of methamidophos with mammalian ChE from several sites in the body have been studied in vivo. In these studies, rats were given methamidophos in a range of doses which allowed the derivation of the LD_{50} of the compound. The protective actions of atropine and pralidoxime were assessed by the changes they produced in the LD_{50}. ChE activity was measured in brain at the time of death in all animals that died promptly from the acute effects of the poison, and in brain, blood, and stomach tissue at 24 hours in all of the survivors.

Acknowledgement: Methamidophos (98% pure) was a gift of Chevron Chemical Co., Richmond, CA.

Other Chemicals: Triton X-100 was obtained from Rohm and Haas, Philadelphia, PA; Sephadex G-25 from Pharmacia Laboratories, Inc., Piscataway, NJ; atropine sulfate from Inland Alkaloid, Inc., Tipton, IN; and 5,5'-dithio-bis-(2 nitrobenzoic acid), pyridine-2-aldoxime methiodide (pralidoxime), Tris and acetylthiocholine iodide from Sigma Chemical Co., St. Louis, MO.

II. Methods.

A. Determination of protection by atropine and pralidoxime against methamidophos poisoning. One hundred and thirty male Holtzman rats were divided into three groups of 40, 40, and 50 rats. These groups were further divided into subgroups of 10. All rats were fasted for 18 hr prior to injections. Forty rats were given methamidophos, 12.5 - 20.0 mg/kg, intraperitonially (i.p.) to determine the acute LD_{50}. The second set of 40 rats was given methamidophos (30-60 mg/kg, i.p.) and each animal was given 10 mg/kg atropine intramuscularly (i.m.) 5 s after injection of the organophosphate. The third set of 50 rats was given methamidophos (30-60 mg/kg, i.p.), and 5 s after the injection each animal was given 60 mg/kg pralidoxime (i.m.). Deaths were recorded for the first 4 and 24 hr. Methamidophos, atropine, and pralidoxime were prepared in water immediately before use. The LD_{50} at 24 hr and its standard error were determined for each group by the method of Miller and Tainter (1944).

The brains of all animals that died were quickly removed and ChE activity determined by the method of Fowler and McKenzie (1967). This is an automated method using acetylthiocholine as substrate. The thiocholine formed by hydrolysis reacts with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) to form a yellow compound which is evaluated colorimetrically.

The survivors of LD_{50} determinations were sacrificed by decapitation 24 hr after methamidophos injections. The brain and stomach were quickly removed and frozen for later ChE determination. Blood samples collected from the cervical area following decapitation were heparinized, and centrifuged to separate cells and plasma. The plasma was refrigerated and assayed for ChE activity 24 hr later.

B. Recovery of tissue ChE activity following poisoning with methamidophos; effects of pralidoxime. One hundred and twenty male Holtzman rats were divided into two groups of 60 each. One group was given 12.5 mg/kg (an LD_{20}) of methamidophos (i.p.). Each animal in the other group received the same dose of methamidophos plus 60 mg/kg pralidoxime (i.m.) approximately 5 s later. Ten additional rats served as controls. The brains of all rats that died were removed and assayed for ChE activity. The surviving rats were divided into subgroups which were sacrificed by decapitation at intervals of 1, 3, 6, 24, and 48 hr after methamidophos was given. The blood plasma, brains, and stomachs of poisoned and control rats were collected and homogenized, and their ChE activity was determined.

III. Results.

A. All rats poisoned with methamidophos exhibited typical peripheral and central signs of cholinergic stimulation including salivation, lacrimation, fasciculations, urination, piloerection, respiratory depression, and convulsions. All animals that died after administration of methamidophos alone did so within 15 min after injection. After 4 hr the survivors showed marked improvement. The LD_{50} for methamidophos (i.p.) was determined to be 15.0 ± 0.7 mg/kg.

Atropine (10 mg/kg) and pralidoxime (60 mg/kg) protected against the lethality of methamidophos, increasing the LD_{50}'s to 60 ± 0.4 mg/kg and 52 ± 4.9 mg/kg, respectively. Those animals that died following treatment with atropine generally showed less frequent tremors and convulsions. All of those treated with atropine that died did so within 15 min of methamidophos administration. Most of the deaths observed in rats given methamidophos plus pralidoxime occurred between the first and second hours. The signs of cholinergic stimulation in the pralidoxime-treated rats were as apparent as those of rats given only methamidophos.

Brain ChE activity at the time of death was markedly depressed after all doses of methamidophos, with activities ranging from 5.3 to 12.4 percent of control. The degree of depression of brain ChE activities following large doses of methamidophos plus pralidoxime or atropine was similar to that seen after smaller doses of methamidophos alone. ChE activities of brain tissue from atropine-treated rats given 45 and 60 mg/kg of methamidophos were significantly higher ($p<0.05$) than those that received pralidoxime and comparable doses of methamidophos (Table 1).

TABLE 1

Brain Cholinesterase Activity of Male Rats at Death Following Methamidophos Poisoning

Dose (mg/kg)	Protectant[a]	Number of Animals	ChE Activity[b] at Time of Death	% of Control Activity ± S.E.M.
12.5	None	19	.81 ± .04	11.5 ± .6
15.0	None	5	.87 ± .15	12.4 ± 2.1
17.5	None	8	.52 ± .08	7.4 ± 1.1
30.0	Atropine	1	.69	9.8
40.0	Pralidoxime	1	.37	5.3
45.0	Atropine	3	.62 ± .01	8.8 ± .01
45.0	Pralidoxime	3	.57 ± .01	8.1 ± .01
60.0	Atropine	5	.64 ± .11	9.1 ± 1.6
60.0	Pralidoxime	7	.45 ± .01	6.4 ± .01

a. Atropine (10 mg/kg, i.m) or pralidoxime (60 mg/kg, i.m.) given approximately 5 seconds after methamidophos (i.p.).

b. Cholinesterase activity is expressed in µMoles of ATCh hydrolyzed/min/g wet weight of tissue. Control brain cholinesterase was 7.3 ± .23.

Twenty-four hours after injection of the various doses of methamidophos, the ChE activity of each tissue was still below control values (Fig. 1).

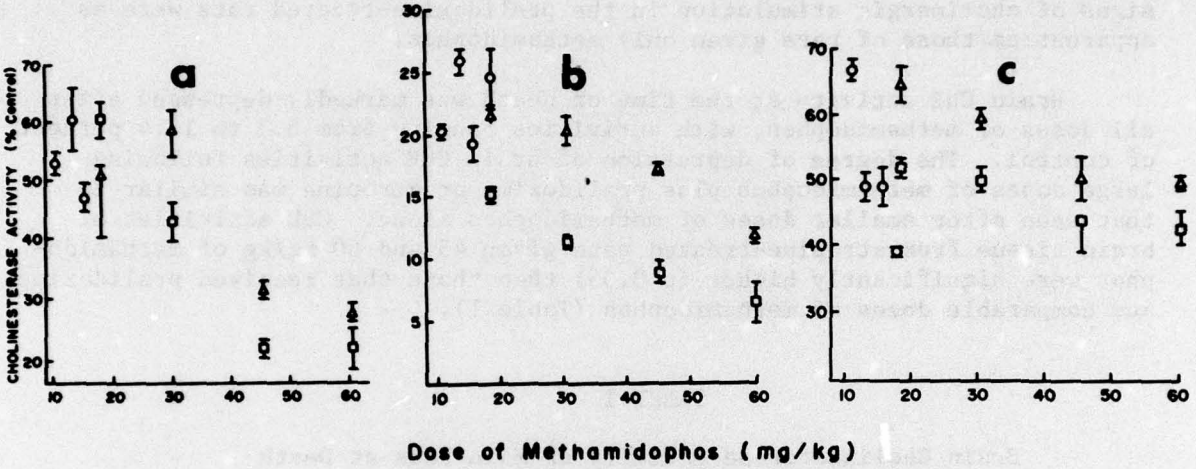


FIG. 1. Cholinesterase activity of plasma (a), stomach (b), and brain (c) from male rats still alive 24 hours after receiving various i.p. doses of methamidophos.

○ = methamidophos, △ = methamidophos plus atropine, 10 mg/kg, i.m.
□ = methamidophos plus pralidoxime, 60 mg/kg i.m. Bar represents one S.E.M.

A linear relationship exists between the degree of depression and the dose of methamidophos administered. At most methamidophos dosage levels, tissue ChE activities of rats given atropine were higher than those from rats given pralidoxime.

B. The rate of return of ChE activity during the first 24 hr was most rapid for the brain (2.1%/hr), compared to the plasma and stomach which were 1.9%/hr and 0.8%/hr, respectively. Forty-eight hours after methamidophos administration, ChE activity in all three tissues had returned to at least 60 percent of control values.

Only slight differences in tissue ChE activity were observed between the unprotected and pralidoxime-protected rats. Although plasma ChE activity was significantly higher in pralidoxime-treated animals for the first 6 hr, the brain and stomach ChE activity was depressed (Fig. 2).

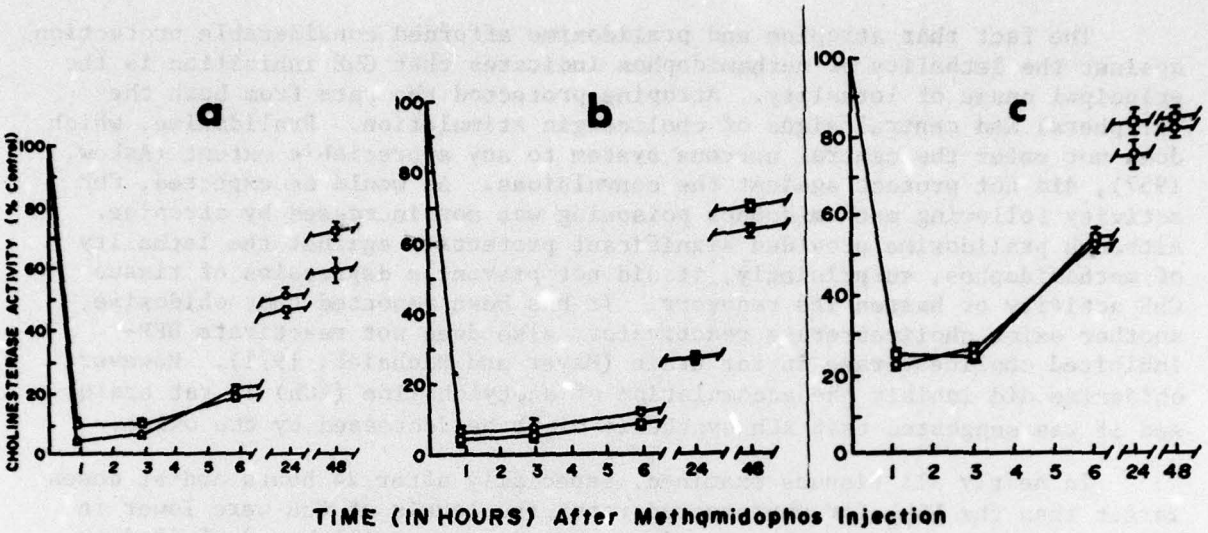


FIG. 2. Recovery of cholinesterase activity of plasma (a), stomach (b), and brain (c) from male rats following injection of methamidophos, 12.5 mg/kg, ip. or methamidophos, 12.5 mg/kg, i.p. plus pralidoxime, 60 mg/kg, i.m.

○= ChE activity following methamidophos. □ = ChE activity following methamidophos plus pralidoxime. Each point is the mean ChE activity ± S.E.M. of tissues from 8 to 12 rats.

IV. Discussion

The toxicity of methamidophos to female houseflies was reported (Quistad et al., 1970) to be approximately the same as that of other potent organophosphorous compounds such as parathion and paraoxon with topical LD_{50}'s of 1.3, 1.0, and 0.5 µg/g for methamidophos, parathion, and paraoxon respectively (Mengle and O'Brien, 1960). Preliminary studies with mice also indicated considerable toxicity to mammals. Our experiments indicate methamidophos is also highly toxic to rats, with an i.p. LD_{50} of 15.0 mg/kg.

Quistad and coworkers (1970) reported that housefly head ChE did not spontaneously reactivate in vitro following methamidophos inhibition and that pralidoxime produced only a partial reactivation. They suggest that the high toxicity may be due in part to rapid aging of the phosphorylated enzyme. This lack of spontaneous reactivation has been reported for housefly head ChE inhibited by such organophosphates as tetraethyl pyrophosphate (TEPP), dichlorvos, and paraoxon (Asperen and Dekhuijzen, 1958; Mengle and O'Brien, 1960). In contrast, in our experiments spontaneous reactivation of methamidophos-inhibited rat tissue ChE's was observed. In agreement with Quistad, however, these experiments indicate that pralidoxime does not effectively reactivate mammalian ChE.

The fact that atropine and pralidoxime afforded considerable protection against the lethality of methamidophos indicates that ChE inhibition is the principal cause of lethality. Atropine protected the rats from both the peripheral and central signs of cholinergic stimulation. Pralidoxime, which does not enter the central nervous system to any appreciable extent (Askew, 1957), did not protect against the convulsions. As would be expected, ChE activity following methamidophos poisoning was not increased by atropine. Although pralidoxime provided significant protection against the lethality of methamidophos, surprisingly, it did not prevent a depression of tissue ChE activity or hasten its recovery. It has been reported that obidoxime, another oxime cholinesterase reactivator, also does not reactivate DFP-inhibited cholinesterase in rat brain (Mayer and Michalek, 1971). However, obidoxime did inhibit the accumulation of acetylcholine (ACh) in rat brain, and it was suggested that ACh synthesis might be decreased by the oxime.

In nearly all tissues examined, especially after 24 hours and at doses larger than the LD_{70} for unprotected rats, the levels of ChE were lower in the pralidoxime- than in the atropine-treated group. All but 2 of 12 data points were significantly different ($p<0.05$). It is difficult to envision why ChE activity should be higher in animals receiving atropine than in those that were given pralidoxime. One possibility is that atropine in uniting with and blocking the receptor for ACh might at the same time shield and protect the enzyme from methamidophos. This presupposes that AChE and the ACh receptor are in close relation to each other, perhaps forming a mosaic in post-synaptic membrane as proposed by Barnard et al. (1971). Some investigators even think that AChE and the cholinergic receptor form a part of the same macromolecule (for review, see Silver, 1974), making this steric or perhaps allosteric effect even a better possibility. A second possibility is that atropine directly inhibits AChE (Long, 1963) and may thus protect the enzyme from methamidophos inactivation during the initial period of methamidophos exposure. It is a well-known fact that a short-acting AChE inhibitor can protect against inhibition by a long-acting inhibitor.

In conclusion, both atropine and pralidoxime should afford protection following exposure to methamidophos, but pralidoxime should not be expected to effect rapid ChE reactivation as it does when given following exposure to a majority of other organophosphorus pesticides.

References

1. Askew, B. M.: Oximes and atropine in Sarin poisoning. BR. J. PHARMACOL. 12:340-343, 1957.

2. Asperen, K. V. and Dekhuijzen, H. M.: A quantitative analysis of the kinetics of cholinesterase inhibition in tissue homogenates of mice and houseflies. BIOCHEM. BIOPHYS. ACTA. 28:603-613, 1958.

3. Barnard, E. A., Wieckowski, J., and Chiu, T. H.: Cholinergic receptor molecules and cholinesterase molecules at mouse skeletal muscle junctions. NATURE 234:207-209, 1971.

4. Fowler, P. and McKenzie, J.: Symposium, Automation in Analytical Chemistry, New York, Mediad, Inc., p. 155, 1967.

5. Long, J. P.: Structure--Activity relationships of the reversible anticholinesterase agents. In, Handbuch der Experimentellin Pharmakologie, pp. 374-427, Springer-Verlag, Berlin, 1963.

6. Lullmann, H., Westermann, F. u. E.: Ubereine, paradoxe atropinwirkung an isolierten organen und ihre statistiche erfassung. NAUNYN-SCHMIEDE-BERG'S ARCH. EXP. PATH. PHARMAK. 215:8-18, 1952.

7. Mayer, O. and Michalek, H.: Effects of DFP and Obidoxime on brain acetylcholine levels and on brain and peripheral cholinesterases. BIOCHEM. PHARMACOL. 20:3029-3037, 1971.

8. Mengle, D. C. and O'Brien, R. D.: The spontaneous and induced recovery of fly-brain cholinesterase after inhibition by organophosphates. BIOCHEM. J. 75:201-207, 1960.

9. Miller, L. C. and Tainter, M. L.: Estimation of the ED_{50} and its error by means of logarithmic-probit graph paper. PROC. SOC. EXP. BIOL. MED. 57:261-264, 1944.

10. Quistad, G. B., Fukuto, T. R., and Metcalf, R. L.: Insecticidal, anti-cholinesterase, and hydrolytic properties of phosphoramidothiolates. J. AGRIC. FOOD CHEM. 18:189-194, 1970.

11. Silver, A.: The Biology of Cholinesterases, pp. 42-47, Amsterdam, North Holland Publishing Company, 1974.

12. Vincent, D. and Parant, M.: Etude de l'action du chlormphenicol (chloromycetine) sur les cholinesterases. C.R. SOC. BIOL. (PARIS)144: 1660-1663, 1950.