Organic Phosphorus Poisoning and Its Therapy

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Organic Phosphorus Poisoning and Its Therapy

With Special Reference to Modes of Action and Compounds That Reactivate Inhibited Cholinesterase

WILLIAM F. DURHAM, Ph.D., AND WAYLAND J. HAYES, JR., M.D., Ph.D., ATLANTA

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Introduction

Organic phosphorus compounds are of considerable interest and importance by virtue of their widespread use as insecticides; their effectiveness in the treatment of myasthenia gravis, glaucoma, and abdominal distention; and their potential application as war gases. These compounds owe their pharmacologic effect primarily, if not entirely, to their ability to inhibit the enzyme cholinesterase with a resultant overstimulation of the parasympathetic nervous system by the acetylcholine which accumulates. Atropine has been the drug of choice for the treatment of organophosphorus poisoning.

This therapy produces relief of symptoms based on blocking the action of excess acetylcholine.

More recently, a number of specific antidotes have been developed which act to repair the basic biochemical lesion in organic phosphorus poisoning by freeing the cholinesterase from its combination with the inhibitor. In the present paper, some of the pertinent background material relative to organic phosphorus poisoning will be reviewed, and the properties and usage of these specific antidotes will be discussed in some detail. Emphasis will be given to those aspects of symptomatology and treatment which might be of most value to the physician faced with a presumptive case of poisoning.

Nature and Physiologic Function of Cholinesterase

There are 2 general types of cholinesterase present in the animal organism. These are: (1) the true, specific enzyme, acetylcholinesterase, which has an almost specific affinity for the naturally occurring substrate acetylcholine, although it will hydrolyze several synthetic esters; and (2) the nonspecific enzyme, pseudocholinesterase, which has the ability to hydrolyze quite a wide range of naturally occurring and synthetic esters in addition to acetylcholine. Acetylcholinesterase occurs in the nervous system, where it is of great functional importance; in muscles; and in glands; and it also occurs incidentally in erythrocytes. The pseudoenzyme is found in the blood plasma and in various tissues, including the central nervous system.
In addition to location and substrate specificity, there are other differences between true and pseudocholinesterases. The latter functions well in the presence of an excess of substrate, while the former is inhibited under this condition. A number of inhibitors show considerable specificity for one type of enzyme. For example, diisopropyl fluorophosphate (DFP) in low concentrations is almost completely specific for pseudocholinesterase.73

It has been suggested that certain direct effects of the anticholinesterase organic phosphorus compounds do not depend on inhibition of cholinesterase.105 Von Kaulia and Holmes142 have reported that patients poisoned by organic phosphorus compounds show abnormalities in the blood-clotting mechanism, but the changes were in both directions from normal and must be considered unconfirmed. Some of the compounds do produce a delayed reaction similar to "Jake-leg paralysis,"11,41,94 but the clinical picture is quite distinct from that of ordinary poisoning. Although any statement about the relationship between cholinesterase inhibition and the classical picture of poisoning must be qualified in terms of the rate of depression, the degree of depression, and perhaps other factors, the evidence for a lack of relationship is indefinite. On the contrary, the evidence for a lack of relationship between cholinesterase inhibition and paralytic effect is much more clear. In spite of these exceptions, it is true that the predominant pharmacologic effect of the organic phosphorus compounds is inhibition of the enzyme acetylcholinesterase.

In order to have an understanding of the action of acetylcholinesterase and of the effect of its inhibition on the animal organism, it is first necessary to look at the physiologic action of acetylcholine. Acetylcholine is the chemical mediator of the parasympathetic nervous system and is necessary for transmission of the nervous impulse (1) from preganglionic fibers to autonomic ganglia; (2) from postganglionic, cholinergic nerves to smooth and cardiac muscle and to secretory cells; (3) from motor nerves to striated muscle; and probably (4) from certain structures within the central nervous system. Normally, in the presence of the usual concentration of cholinesterase, the acetylcholine formed during the process of transmission is hydrolyzed almost instantly so that the synapse is again ready to transmit a physiologic impulse. A small abnormal accumulation of acetylcholine at the synapse or myoneural junction produces an abnormal increase in function (e.g., fasciculation of muscle), while greater accumulation rapidly produces a decrease in function (e.g., paralysis). A decrease in cholinesterase activity is accompanied by an accumulation of acetylcholine. The symptoms caused by organic phosphorus poisoning are, therefore, very similar to those resulting from overstimulation of the parasympathetic nervous system.

**Symptoms of Organic Phosphorus Poisoning**

Absorption of the organic phosphorus anticholinesterases may occur through the lungs, gastrointestinal tract, or skin. Absorption of these materials is more rapid and more complete through the former 2 routes than through the latter. Respiratory exposure may be of predominant importance wherever there is a sufficient concentration of vapor or of aerosol fine enough to inhale. However, in many agricultural and public health usages, workers get contamination predominantly on their skins.13,30,42 The oral route of exposure occurs in accidental poisoning (particularly of children), in murder, and in suicide. Ingestion has not been considered of importance in occupational exposure; but a case has been reported in which a parathion sprayman became severely poisoned, presumably from eating a candy bar which he had been carrying for 6 hours in an open outside pocket of his work clothes.120

Although many of the organic phosphorus compounds have a high acute toxicity, agricultural residues of these materials on food have not been a problem, due to their rapid breakdown and the fact that they are not stored in the animal body.40 Direct contamination of food by concentrated formulations
ORGANIC PHOSPHORUS POISONING

of these insecticides during shipment has been the cause of several outbreaks of poisoning in other countries.

The symptom picture in organic phosphorus poisoning may vary in severity, in rapidity of onset, in duration, and in range, depending upon the route and the magnitude of exposure. Minor exposure to a vapor or aerosol of a direct inhibitor of cholinesterase may produce local effects on the eye or respiratory system through local absorption and without systemic effect. The optic effects consist of miosis, a sensation of pressure in or behind the eye, headache, and conjunctival hyperemia. Unilateral manifestation of these optic effects has been implicated in visual difficulties experienced by pilots applying these materials. The local effects on the respiratory tract involve increased secretion, a feeling of tightness in the chest, and occasionally wheezing. Localized massive dermal exposure can lead to muscular fasciculation and sweating confined to the area of absorption.

Systemic effects may follow absorption by any route. If there has been adequate exposure to a vapor or aerosol, the local respiratory effects already described will appear, but they will be rapidly followed by more severe optic and respiratory distress and by systemic manifestations. Dixon has pointed out that too much reliance should not be placed on miosis as a diagnostic sign, since some poisoning cases, at least early in their course, do not exhibit miosis, and a few even have mydriasis.

In systemic poisoning, the muscarine-like effects are usually first to appear. They include anorexia, nausea, sweating, epigastric and substernal tightness (probably due to cardiospasm), heartburn, belching, and tightness in the chest. The sequence of symptoms varies somewhat with the route of exposure —gastrointestinal effects usually being earliest after ingestion; sweating, and at times muscular fasciculations, after dermal exposure; and respiratory effects after inhalation. More severe exposure by whatever route produces abdominal cramps, increased peristalsis, vomiting, diarrhea, salivation, lacrimation, profuse sweating, pallor, and dyspnea. In some subjects there is audible wheezing. More severe signs and symptoms include involuntary defecation and urination, excessive bronchial secretions, and (according to some authors) pulmonary edema.

Nicotine-like effects appear usually after the muscarine-like effects have reached moderate severity. These include muscle twitching, fasciculations, and cramps. At about the same time, there appears increased fatigability and mild, generalized weakness which is increased by exertion. Extensive exposure produces severe weakness, including weakness of the muscles of respiration. There may be a mild or moderate elevation of blood pressure.

The central-nervous-system effects include tension, anxiety, restlessness, giddiness, and emotional lability. Late effects include insomnia, excessive dreaming, and occasionally nightmares. Greater exposure produces headache, tremor, drowsiness, difficulty in concentration, slowness of recall, and confusion. There may be withdrawal and depression. In the absence of symptoms, there is no change in the electroencephalogram (EEG). Mild symptoms are accompanied by a slight diminution in potential, and moderate symptoms are accompanied by irregularity of rhythm, variation and increase of potential, and bursts of abnormal waves more or less reminiscent of waves seen in epileptics. Lethal or near-lethal doses produce ataxia, slurring of words, multiple repetition of the last syllable of words, coma, areflexia, Cheyne-Stokes breathing, and finally, respiratory arrest.

The cause of death may usually be attributed to interference with respiration. Animal experiments have proved that the anticholinesterase organic phosphorus compounds interfere with respiration in at least 4 ways, including bronchoconstriction, excessive respiratory secretion, failure of the muscles of respiration, and depression of the respiratory center. Although the respiratory center is at first stimulated by anoxia of whatever cause, it is rapidly depressed by continued anoxia. The severe bronchial con-
striction seen in animals following exposure to parathion or nerve gas has not been seen in man. The difference may depend on the different amount of bronchial musculature in different species.

The compounds also produce bradycardia and various degrees of A-V block. If these effects are prevented by atropine, and if the animal is prevented from a respiratory death by artificial respiration, it will eventually succumb to a sufficient dose through dysfunction of the heart. This dysfunction will take the form of ventricular fibrillation if atropine should be administered in the presence of severe anoxia. Even if atropine is given correctly, bradycardia and perhaps impaired contractility appear shortly before asystole in the animal maintained by artificial respiration. Unanesthetized dogs, unlike anesthetized ones, exposed to sarin by various routes generally show bradycardia, increase in pulse amplitude, decrease in oxygen tension, increase in carbon dioxide tension, and fall in arterial blood pH before changes in respiratory rate and volume are evident.

The local effects of organic phosphorus exposure begin within a few minutes after exposure and last for periods ranging from several hours to a day, except that miosis may last 2 to 5 days or occasionally longer. Moderate systemic effects begin within about half an hour after respiratory exposure, three-quarters of an hour after oral exposure, and 2 to 3 hours after dermal exposure. With sufficient exposure, the onset of symptoms is essentially instantaneous, with death in a few minutes. Moderately severe symptoms may not reach their maximum until 4 to 8 hours after onset; they diminish over a period of 1 to 6 days. However, EEG changes may persist for 11 to 18 days.

**Mechanism of Inhibition of Cholinesterase**

In recent years, much has been learned about the molecular forces involved in the interaction of acetylcholinesterase both with its natural substrate and with organic phosphorus inhibitors. Wilson and Bergmann have postulated that the surface of the enzyme protein contains 2 active centers, an "anionic site" and a "cationic or esteratic site." The former, by means of coulombic forces, attracts the positively charged quaternary nitrogen atom of acetylcholine and thereby fixes and orients the substrate in a proper position so that the esteratic site can exert its hydrolytic effect. The esteratic site is thought to consist of a hydrogen-bonding group which anchors the ester end of the acetylcholine molecule. It has been suggested that the ester-anchoring group may be the iminazole ring of histidine and that any phosphoryl group which becomes attached here is subsequently transferred to an adjacent serine moiety of the enzyme molecule by a $\text{N}\to\text{O}$ shift. It is thought that the effect of change in pH on the hydrolytic activity of this enzyme is mediated through changes in ionization at these 2 sites.

In the following stage of the physiologic reaction, the acetylcholine is split into choline which is set free and the acetyl moiety which remains affixed to the enzyme. However, the acetylated enzyme is unstable and easily breaks down to form active enzyme and acetic acid.

The reaction between enzyme and organic phosphorus inhibitor is thought to involve only the esteratic site, except with the phosphorylcholines that are used as laboratory tools only and need not be discussed further. This center is phosphorylated to form a complex which is, at least by contrast with the acetylated enzyme, quite stable. The phosphorylation is thought to proceed in 2 stages, as discussed in detail below under "Chemical and Physiologic Properties of Oximes." The stability of this phosphorylated enzyme accounts for the fact that organic phosphorus compounds are inhibitors rather than substrates for cholinesterase. During this inhibition reaction, the anionic site remains uninvolved.

**Measurement of Cholinesterase Activity and Its Relationship to Symptomatology**

Cholinesterase activity can be determined by any of several methods based on: (1) di-
rect or indirect measurement of the acetic acid or other moiety released by hydrolysis of acetylcholine or other esters, or (2) direct measurement of acetylcholine remaining after partial hydrolysis.

The electrometric method of Michel together with a micromodification of this technique for use with capillary blood samples is probably the most widely used method. There are also available colorimetric, titrimetric, and manometric procedures. The colorimetric screening method of Limperos and Ranta is adaptable to the approximate determination of large numbers of samples under field conditions. Other procedures involve use of the Hestrin method for measurement of acetylcholine.

The restoration of plasma or of plasma and red blood cell cholinesterase activity may be increased by plasma or whole blood transfusions in animals without any effect on the symptoms of poisoning or appreciable reduction in the susceptibility to further exposure.

Although the acetylcholinesterase of red blood cells and the more general esterase of the plasma are not directly related to the signs and symptoms of organic phosphorus poisoning, these enzymes are inhibited in a manner essentially parallel to the inhibition of acetylcholinesterase of nerve, muscle, and gland. The blood enzyme levels, and particularly that of the erythrocytes, may be used as an index of tissue enzyme levels. The erythrocyte cholinesterase level has been shown to correlate rather closely with the activity of this enzyme in the brain during both poisoning and recovery in the rat. Following a single exposure, maximum depression of blood cholinesterase occurs within a few hours. The degree of depression varies with the amount of absorption, the logarithm of the fraction of enzyme inhibited being proportional to the amount of toxicant absorbed in connection with essentially instantaneous dosage. Of course, absorption is influenced by route, often being prolonged for hours or even days after skin exposure.

Multiple exposures over a brief period are partially cumulative in their effect. Experience suggests that small multiple exposures over an extended period are not indefinitely cumulative in their effects but that the cholinesterase level reaches a plateau. There is some evidence that the rate of inactivation of tissue cholinesterase, as well as the degree of inhibition, may influence the level of tissue enzyme activity at which symptoms begin. The cholinesterase activity of the red blood cells may be gradually depressed to near zero by repeated exposure over a period of weeks without systemic symptoms necessarily ensuing, or without any relation to the severity of symptoms that occur.

Following exposure, the blood enzymes, especially the erythrocyte cholinesterase, remain at a low level of activity for some time after the disappearance of symptoms. This lag is thought by some to indicate that cholinesterase activity is restored more rapidly in the tissues than in the blood, although the tissue regeneration rate is not fully known. Chronic-feeding and certain other studies indicate that the phenomenon may be accounted for by tolerance rather than by a difference of recovery rate for different enzymes. The adaptation to constant concentrations of acetylcholine that has been demonstrated to occur in ganglia may well occur in other parts of the nervous system. This adaptation almost certainly contributes to the lack of parallelism between symptomatology and cholinesterase level.

It is true that effects of doses of anticholinesterase compounds near the fatal level are partially cumulative if the doses are repeated at relatively brief intervals. Thus, Callaway and Davies have found in rabbits and guinea pigs that lowering the blood cholinesterase level to 50% of normal by either a single dose or repeated doses of tetraethyl pyrophosphate (TEPP) or sarin results in an increased susceptibility of the animal to these agents.

Plasma cholinesterase is formed by the liver. Following cessation of exposure, plasma enzyme activity is increased by about 13% of original activity during the first day, and more slowly thereafter, so that 30 to 40
days are required to reach the normal pre­
exposure level.

Once fully inhibited, the enzyme content of a particular erythrocyte is not regenerated. Rather, in the absence of treatment with oximes, the rate of regeneration of red­blood-cell cholinesterase reflects the replace­ment of red corpuscles in the circulation and thus requires 90 to 100 days to return to original activity after nearly complete depres­sion. Regeneration occurs at a regular rate of about 1% of normal per day.

Barstad 12 concluded that respiratory func­tion should be only slightly impaired by a peripheral cholinesterase inhibition ap­proaching 90% and that consequently a rather slight degree of cholinesterase reac­tivation at the critical site should be sufficient to relieve the peripheral failure of respira­tory movements during poisoning with anti­cholinesterase agents.

Measurement of Metabolites and Their Relationship to Symptomatology

Exposure to those organic phosphorus compounds which, on hydrolysis, form p-nitrophenol or one of its congeners can be estimated by determination of these phenolic compounds in urine.45 Although applicable to a restricted group of compounds, this test has proved to be a more sensitive measure of absorption than is cholinesterase inhibition.4 A urine test based on excretion of organically-bound phosphorus would be desirable, since metabolites of this type are, of course, common to all the compounds in this group.

Use and Action of Atropine and Other Nonspecific Antidotes

Until recently, the most important factors influencing survival after otherwise lethal cholinesterase inhibition appear to be prompt administration of atropine in sufficient dosage and artificial respiration, if required.61

The suggested dose of atropine is 2 mg, initially, and as much as 6 mg may safely be given within 10 minutes or more without medical supervision. Individuals poisoned by anticholinesterase organic phosphorus compounds have an increased tolerance for atro­pine. Furthermore, a single dose of as much as 10 mg of atropine has been inadvertently administered intravenously to normal adults without endangering life, although it has, of course, produced very marked signs of over­dose. In the presence of severe anticholines­terase poisoning, 40 mg of atropine may be given in a day without producing symptoms of overatropinization. The low toxicity of atropine and its effectiveness in the treatment of anticholinesterase intoxication have been discussed by Gordon and Frye.65

Severe symptoms of organic phosphorus poisoning should be treated by the physician with the intravenous injection of 2 to 4 mg of atropine. The effects of intravenous atro­pine begin in 1 to 4 minutes and are maximal within 8 minutes. If muscarine-like symp­toms are not relieved, and if signs of atropin­ization (dry, flushed skin and tachycardia) do not appear, the intravenous injection of atropine in doses of 2 mg should be repeated at 5- to 10-minute intervals until symptoms are relieved or signs of atropine overdosage appear. A mild degree of atropinization should be maintained in all cases for 24 hours and in severe cases for at least 48 hours.

Atropine should not be given to an anoxic patient because of the danger of producing ventricular fibrillation. In the cyanotic pa­tient, artificial respiration, oxygen, or other indicated measures should be carried out first to correct the anoxia, and then atropine should be given.

Atropine should not be administered for preventive purposes in persons who antici­pate exposure to anticholinesterase agents. Its use in this manner may mask the early occurrence of signs and symptoms of intoxi­cation and allow the patient to expose himself to dangerous levels of the toxicant without warning. Wills 145 has noted that animals given atropine before respiratory exposure to sarin vapor were somewhat more sus­ceptible to the poison than controls given no antidote. The ineffectiveness of atropine in this situation was interpreted as being due to its action to prevent the bronchoconstric-
tion which otherwise occurs to some degree after inhalation of nerve gas vapor (see "Symptoms of Organic Phosphorus Poisoning"), therefore resulting in greater inhalation and absorption from the respiratory tract. Patients who are sick enough to receive atropine should be hospitalized and kept under careful observation for 24 hours; in some instances, a marked improvement has been noted after atropine followed later by deterioration of the patient's condition, requiring further treatment promptly.\(^7\)

Ocular symptoms produced by local absorption do not respond to the systemic administration of atropine but are relieved by the local administration of 2% homatropine or, if severe, by the local administration of 0.5% or 1% atropine.\(^7\) Parenteral atropine may relieve miosis, but the effect is both irregular and transient. If local ocular effects are present, the size of the pupils obviously cannot be used as an indicator of systemic poisoning or as a gauge for atropine dosage.

If convulsions interfere with artificial respiration and are not relieved by intravenous atropine, the patient may be given trimethadione (Tridione), a barbiturate, or ether. Trimethadione may be given intravenously or intramuscularly, in doses of 1 gm., repeated if necessary. Morphine should not be given. The action of succinyl choline (Suxamethonium) increases the effect of the anticholinesterase agents, and thus the use of this relaxant is contraindicated (see "Chemical and Physiologic Properties of Oximes").

Results of treatment using transfusion with normal blood or even intravenous infusion of purified cholinesterase have, in general, been disappointing.\(^4\) It is doubtful whether these materials provide any active enzyme to important sites in the nervous tissue. Their action in combining with any circulating poison is too little and too late to be of value in reversing the pathology associated with poisoning.

It is important to understand that, while atropine is effective as an antidote, it has no effect on the fundamental biochemical lesion involved in poisoning by anticholinesterase compounds. Atropine merely serves to block certain actions of the excess acetylcholine already accumulated. Atropine has no action on the nicotinic effects of acetylcholine, including the neuromuscular block that leads to muscle weakness, and may finally end in death due to paralysis of the respiratory muscles.\(^4\) Although atropine specifically blocks the muscarinic actions of acetylcholine, it is less effective in blocking certain muscarinic actions (such as those on the intestine and urinary bladder) than others (such as cardiac slowing and sialorrhea). The effects of anticholinesterases on the central nervous system are reversed by atropine, and the increased electrical activity of the brain, as shown by the EEG, is returned to normal.\(^1\)

Tests with various synthetic parasympatholytic agents, including l-N-butylscopolammonium bromide (Buscopan),\(^3\) have not shown any of these compounds to be significantly more effective than atropine in antidotal action.

**Development of Specific Antidotes**

Numerous investigators have sought to find agents that would interfere in the combination of the organic phosphorus compound with cholinesterase or hasten the destruction of the inactive phosphorylated enzyme complex. The development of specific antidotes of this type has been reviewed by Davies and Green.\(^3\) The role of oximes in the treatment of anticholinesterase poisoning has also been reviewed by Wills\(^1\) and by Verhulst and Page.\(^1\)

Probably the usage of physostigmine (eserine) and neostigmine\(^2\) represents the earliest record of such attempts. It was found that, instead of an expected summation or potentiation of effect occurring between DFP and physostigmine or neostigmine, prior administration of one of the latter agents protected rats against lethal doses of DFP. It was postulated that this antidotal effect was due to the formation between the physostigmine or neostigmine and cholinesterase of an unstable bond which served to protect the active site on the enzyme from attack by the irreversible inhibitor, DFP. The effectiveness of these particular agents

*Durham—Hayes*
is strictly prophylactic, and they have no antidotal effect when given after the poison. An additive rather than an antagonistic action was noted when physostigmine was given after DFP or parathion.

Somewhat later, Wilson reported that choline and hydroxylamine were effective in reversing the combination between cholinesterase and certain organic phosphorus inhibitors. Wagner-Jauregg and his coworkers used certain metal salts and chelates to reactivate DFP-inhibited enzyme.

The most promising development, however, is that associated with the hydroxamic acids and oximes. As pointed out above, it has been shown that the organic phosphorus compounds inactivate cholinesterase by forming a firm bond with the enzyme. This combination is commonly spoken of as "irreversible" in contrast to the combination of cholinesterase with physostigmine or neostigmine, which can easily be reversed by dilution or dialysis. Actually, "irreversible" must be regarded here as a relative term. In fact, TEPP-inactivated enzyme spontaneously recovers some of its activity in vitro if allowed to stand in water for a sufficiently long time. It is more difficult to reactivate DFP-inhibited enzyme.

It would be expected that reagents (such as esters or acids) capable of making a nucleophilic attack on the phosphorus atom of the inhibitor-enzyme complex could regenerate the enzyme. Theory would further predict that presence within the reactivating molecule of a cationic structure, such as the ammonium radical, would facilitate this reaction by acting, as does the nitrogen atom in acetylcholine, to properly position the molecule for its reaction with the enzyme. The hydroxamic acids and oximes fulfill these requirements imposed by theory by having both nucleophilic and cationic ammonium groupings in the molecule.

The mechanism of action of oximes in regenerating inhibited cholinesterase is thought to consist of an initial direct combination between the organic phosphorus-inhibited enzyme and the hydroxamic acid, followed by a reaction in which the organic phosphorus moiety is split off and hydrolyzed. The hydroxamic acid residue undergoes a further reaction to regenerate the active enzyme. The important thing from a practical standpoint is that the hydroxamic acid has a greater affinity for the phosphorus moiety than does the enzyme.

Epstein and Freeman have reported the results of a study of the toxicity and prophylactic and therapeutic efficiency of a series of 15 hydroxamic acids in nerve-gas poisoning in mice. Other workers at the Army Chemical Center have made and tested 22 oximes in this regard. O'Leary et al. have tested a number of oximes both singly and in various combinations.

Of the hydroxamates and oximes tested by various workers, the outstanding compounds are 2-pyridine aldoxime methiodide (2-PAM iodide) and related salts. These compounds combine the features of low mammalian toxicity and good prophylactic and therapeutic efficiency against organic phosphorus poisoning.

Details regarding usage of oximes, and particularly of 2-PAM salts, in the treatment of anticholinesterase poisoning are considered in succeeding sections of this review.

2-Pyridine aldoxime methyl methanesulfonate (P2S), which is the methane sulfonate salt corresponding to 2-PAM, has recently been shown to be just as effective therapeutically as 2-PAM iodide and to possess the advantage of greater water solubility. The methochloride salt of 2-pyridine aldoxime (2-PAM chloride) is more stable and more water soluble than 2-PAM iodide. The methochloride salt is now commercially available in the United States as an investigational drug under the trade name Protopam.* The lactate salt has been studied by O'Leary et al.

In comparing the effects of these various salts of 2-pyridine aldoxime, it is necessary to take into account the variation in the

* From Campbell Pharmaceuticals, Inc., 121 E. 24th St., New York 10. (The use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service.)
amount of actual free base in the dosage administered. For example, 1.0 gm. of 2-PAM chloride is approximately equal to 1.5 gm. of 2-PAM iodide with regard to content of the active free-base, 2-pyridine aldoxime.

British workers \textsuperscript{5,23,128} have proposed monoisonitroacetone (MINA) and diacetylmonoxime (DAM) as being superior to 2-PAM in combating organic phosphorus poisoning. DAM is less toxic than 2-PAM.\textsuperscript{6} These oximes reactivate sarin-inhibited cholinesterase in vitro and are effective antidotes for sarin poisoning in rats. They reported that certain monoximes and dioximes of polymethylenebispyridinium compounds were from 15 to 52 times as potent as 2-PAM. Poziomek et al.\textsuperscript{119} have also synthesized a number of compounds in this series. The outstanding antidotal compound from this group is 1,1'-trimethylene-bis(4-formyl-pyridinium bromide) dioxime (TMB-4).\textsuperscript{15,52,79-81} These other oximes have not been studied so thoroughly as the salts of 2-PAM.

Another type of specific antidote which should be mentioned includes compounds which increase the hydrolytic activity of cholinesterase. Among the compounds which have been shown in vitro to possess this sort of activity are tryptamine,\textsuperscript{50} certain analgesics,\textsuperscript{53} and other compounds.\textsuperscript{55,72,139} Most of this work has been carried out with plasma cholinesterase, although some studies using the specific enzyme have been done.\textsuperscript{110,134,137} There is, at present, little or no indication that these agents would be of practical value in the treatment of poisoning.

Ball and his co-workers \textsuperscript{10} found that rats which had been given a large but sublethal dosage of aldrin were able to withstand amounts of parathion that would ordinarily be lethal. This protective effect of aldrin was accompanied by an increased plasma cholinesterase level. Similar results have been noted with other chlorinated hydrocarbon insecticides, including chlordane, dieldrin, DDT, heptachlor, and the \( \alpha \) and \( \gamma \) isomer (lindane) of benzene hexachloride (BHC).\textsuperscript{29,115} Other known hepatotoxic agents (such as carbon tetrachloride) have a similar effect. The transitory rise in serum esterase apparently is a temporary consequence of the tissue damage occurring in the liver. Since the protective effect has been noted for a direct inhibitor (TEPP) as well as for compounds which must undergo biotransformation in order to become toxic, a decreased activating ability of the liver would not seem to be the mechanism of action. The most likely possibilities appear to be an increased level of phosphorylatable enzyme which competes with the true cholinesterase for the inhibitor, or an increased level of hydrolyzing enzyme which aids in the degradation of the anticholinesterase. O'Brien\textsuperscript{116} considers the latter possibility to be more likely.

Many of the organic phosphorus compounds (including parathion, the other phosphorothioates, and some phosphoroamidates) become potent anticholinesterase agents only after bioactivation by the liver.\textsuperscript{116} It has proved possible to protect animals from compounds of this type by blocking the activating enzymes present in the liver. Thus, Davison\textsuperscript{36} was able to protect mice from poisoning by octamethyl pyrophosphoramide (OMPA) but not by parathion through prior administration of the liver microsome inhibitor SKF 525A (\( \beta \)-diethylaminoethyl diphenylproplacetate hydrochloride). O'Brien and Davison\textsuperscript{117} studied other liver microsome inhibitors, but found SKF 525A to be the best of 12 agents tested. They confirmed the failure of SKF 525A to protect against parathion but found that it antagonized Guthion insecticide poisoning. The lack of effectiveness of SKF 525A against parathion poisoning has not been satisfactorily explained. The failure does not seem to represent a general ineffectiveness against phosphorothionates, since the \( \text{LD}_{50} \) of Guthion was increased.

In connection with this effect on the enzyme system which activates the organic phosphorus insecticides, it is interesting to note that compounds are also known which inhibit the enzyme systems that detoxify the organic phosphorus compounds. Among the compounds producing this effect are certain of
the organic phosphates themselves. Thus, when given in combination, the resultant toxic action of certain of the organic phosphorus compounds is greater than would be expected on the basis of simple additive effect. The potentiation was first reported for malathion plus ethyl-\(p\)-nitrophenyl thionobenzene phosphonate (EPN), while the maximum synergistic effect (88 to 134 times) noted has been observed with malathion plus triorthotolyl phosphate TOTP. The mechanism by which potentiation occurs has been elucidated and appears to involve the interference of one compound with the metabolism of the

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<tr>
<th>Chemical Name</th>
<th>Common Name</th>
<th>Structural Formula</th>
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| Hydroxyl amine | 2-PAM or 2-PAM iodide | \[
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\text{N} & \\
\text{N} & \\
\end{align*}
\] |
| 1,1'-trimethylene bis(4-formyl-pyridinium bromide) dioxime | TMB-4 or TMB-4 dibromide | \[
\begin{align*}
\text{Br} & \\
\text{Br} & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\end{align*}
\] |

Chemical name, common name, and structural formula of oximes used as antidotes in poisoning by anticholinesterases.

Vol. 5, July, 1962
Toxicity of Oximes to Experimental Animals

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Sex</th>
<th>Route</th>
<th>LD_{50} mg/kg</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>2-PAM iodide</td>
<td>Mouse</td>
<td>Mixed</td>
<td>Intraperitoneal</td>
<td>136± 6 92</td>
<td></td>
</tr>
<tr>
<td>2-PAM iodide</td>
<td>Mouse</td>
<td>Mixed</td>
<td>Intraperitoneal</td>
<td>23±10 102</td>
<td></td>
</tr>
<tr>
<td>2-PAM iodide</td>
<td>Mouse</td>
<td>Unstated</td>
<td>Intrapertoneal</td>
<td>209±20 160</td>
<td></td>
</tr>
<tr>
<td>2-PAM iodide</td>
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<td>Unstated</td>
<td>Subcutaneous</td>
<td>140±15 160</td>
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<tr>
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<td>Unstated</td>
<td>Intravenous</td>
<td>145± 8 118</td>
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<tr>
<td>2-PAM sulfonate</td>
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<td>Unstated</td>
<td>Intravenous</td>
<td>118±13 118</td>
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<td>Intravenous</td>
<td>115± 3 118</td>
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<tr>
<td>2-PAM chloride</td>
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<td>Mixed</td>
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<td>55± 6 118</td>
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<tr>
<td>TMB-4 dibromide</td>
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<td>Intravenous</td>
<td>57± 1 118</td>
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</tr>
<tr>
<td>TMB-4 dichloride</td>
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<td>Unstated</td>
<td>Intravenous</td>
<td>57± 1 118</td>
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</tr>
<tr>
<td>TMB-4 dichloride</td>
<td>Rabbit</td>
<td>Mixed</td>
<td>Intravenous</td>
<td>44 118</td>
<td></td>
</tr>
</tbody>
</table>

Second 27,28,109 Seume and O'Brien 133 have reported that inhibition of carboxylesterase and carboxyamidase is important in potentiating these compounds.

A potentiating effect between certain of the organic phosphorus insecticides and some phenothiazine-derived tranquilizers has been found to occur in rats 64 and has been suspected to occur in man.8

It has been noted that the signs and symptoms of parathion poisoning were intensified and prolonged in a patient who was given succinylcholine for control of convulsions.120

Chemical and Physiologic Properties of Oximes

The chemical structure of 2-PAM iodide is shown in the Figure. The similarity of the aliphatic portion of this molecule to hydroxylamine is apparent. Pure 2-PAM iodide is a yellow, crystalline solid which decomposes at 219°C. It is soluble in water up to about 5% and practically insoluble in ethyl alcohol. The methanesulfonate salt (P2S) is soluble in water up to about 40%.84 The chloride is soluble in water to the extent of 1.0 gm. in less than 1.0 ml.

The toxicity to laboratory animals of various oximes as determined by a number of different investigators is summarized in the Table. It can be seen that the estimate of the intraperitoneal LD_{50} of 2-PAM iodide to mice ranges from 136 to 223 mg. per kilogram, and the intravenous LD_{50} ranges from 44 to 145 mg. per kilogram for mice and rabbits. The toxicity of the compound, while important, is not especially great. When lethal doses are given, death usually occurs within 10 to 20 minutes and is due to respiratory failure. Generalized clonic convulsions and gasping respiratory movements are seen. Generalized skeletal muscle tremors continue for a few minutes after cessation of respiration. The similarity of this symptom picture to that seen in organic phosphorus poisoning leads one to suspect that the mechanism of toxic action of 2-PAM may involve its observed capacity to inhibit cholinesterase in excess dosages (see further details on page 34).

In man, side-effects from the oximes 2-PAM iodide, 2-PAM chloride, and diacetylmonoxime (DAM) have been minimal in normal subjects and practically nonexistent in people who were poisoned.65,69,87 Rapid intravenous injection of 2-PAM iodide has produced transient mild weakness, diplopia, blurred vision, dizziness, impairment of accommodation, and occasionally headache, nausea, and tachycardia.87 A few patients given 2-PAM iodide have complained of a bitter taste that no doubt resulted from the iodine moiety of the molecule; 2-PAM iodide has also produced an allergic rhinitis and a feeling of fatigue in the jaws.113

Intravenous injection of DAM has caused a burning sensation at the site of injection radiating up the vein, giddiness, drowsiness, a sensation of warmth and tingling in the abdomen and chest, tachycardia, mild postural hypotension,68 and occasionally bitter taste, paresthesias and decreased position sense in the extremities, decreased sweating, transient loss of consciousness, clonic move-
ments of the head, and decreased amplitude of the electroencephalogram and of the T-wave segment of the electrocardiogram.87 Many of the oximes liberate cyanide in the body and also on long standing in vitro.8,111 When there is doubt concerning the integrity of a 2-PAM preparation, particularly solutions of old or uncertain age, a simple test for free cyanide should be carried out.

It should be noted that 2-PAM salts have at least 3 actions which may be of importance in their effect on animals poisoned by organic phosphorus compounds. These actions are:

1. Reactivation of inhibited cholinesterase.
2. Reaction with and inactivation of the organic phosphorus molecule.
3. Inhibition of cholinesterase, especially in excess dosage.

The reactivation of inhibited cholinesterase is the dominant effect of 2-PAM in poisoned animals. In vitro, 2-PAM is a very powerful reactivator. At a concentration of \(10^{-5}\)M., 2-PAM iodide reactivates as much as 80% of alkyl phosphate-inhibited enzyme within 1 minute.154,31,23 The diisopropyl phosphor- ylated enzyme is reactivated more slowly and less completely than the diethyl substituted cholinesterase.77 In a comparative in vitro study of 2-PAM iodide, DAM, and mono-isonitroacetone (MINA), Cohen and Wiersinga 24 found that 2-PAM iodide was the most efficient reactivator, followed closely by MINA. The reactivation by DAM was slow when compared with those of the 2 other oximes. In a later paper, Cohen and Wiersinga 25 theorized that the antidotal efficacy of DAM was primarily due to its ability to inactivate the organic phosphorus molecule rather than its capacity to regenerate inhibited cholinesterase. Rajapurkar and Panjwani 124 suggested that DAM had an antiacetylcholine effect, like that of atropine. Their hypothesis was based on study of ciliary movement in the frog esophagus. This preparation has been shown to contain the acetylcholine-cholinesterase-choline acetylase system, but no nervous tissue. DAM produced slowing of the ciliary movement similar to that produced by atropine or curare. The action was the same in eserinized or normal cilia. Thus, apparently neither a central mechanism nor a reactivation of inhibited enzyme was responsible for the effect of DAM in this instance.

Kewitz 90 has shown that 2-PAM iodide reactivates the esterase activity of diaphragm in the living animal poisoned with paraoxon or DFP, but not OMPA. These results parallel those obtained in vitro with the same compounds and afford good evidence that the antidotal properties of 2-PAM depend upon reactivation of inhibited cholinesterase. Cohen and Wiersinga 25 reported that both MINA and 2-PAM iodide regenerated diaphragm cholinesterase in vivo in sarin-poisoned rats. Inhibited brain cholinesterase was reactivated by MINA only.

Some workers 7,78 have not been entirely satisfied with this explanation for the antidotal efficacy of 2-PAM, and, in support of their view, point out that the therapeutic efficacy and in vitro cholinesterase reactivating power of the oximes do not correlate very closely. One would do well to remember, however, that the other 2 actions of oximes (numbers 2 and 3 above) must also be considered and may explain the observed lack of correlation.

In contrast to the marked rise of enzyme activity in blood and muscle following the administration of 2-PAM, little or no effect has been noted on inhibited brain cholinesterase.78,128,90,91,25 This failure of 2-PAM to reactivate brain cholinesterase has usually been ascribed to a supposed inability of the oxime to pass the blood-brain barrier. In earlier work, Koelle and Steiner 96 had shown that quaternary nitrogen compounds penetrate the blood-brain barrier with difficulty. However, even when administered intracranially, P2S had no effect on the central actions of sarin. Holmstedt 84 considered that the observed failure of 2-PAM iodide to restore respiration depressed by anticholinesterase was due to its inability to penetrate the blood-brain barrier. Jager et al. 88 found low concentrations of 2-PAM iodide in the brain of 2 rabbits infused with this antidote. Wilson 152 has attempted to overcome this difficulty by using the fat-soluble dodecanoic acid analogue of 2-PAM with only partial success. In contrast to these results, Rosen-
berg\textsuperscript{126} has noted in vivo reactivation of brain cholinesterase by 2-PAM iodide after paraoxon poisoning in the rabbit. Rosenberg considered that the differences between his results and those of others cited above might be due to species variation or to the use of different extraction procedures. Differences in dosage levels of 2-PAM may also have been a factor, since Rosenberg gave very high dosages (up to 250 mg. per kilogram).

The marked effectiveness of 2-PAM iodide, 2-PAM chloride, and TMB-4 in poisoning by Phospholine Iodide in mice is considered to be related to the fact that phospholine, in contrast to other organic phosphorus cholinesterase inhibitors, contains a quaternary nitrogen atom, and thus, like the quaternary oximes, fails to pass the blood-brain barrier.\textsuperscript{911} TMB-4 did not reactivate brain cholinesterase activity in rats poisoned with DFP or sarin.\textsuperscript{51} Jager et al.\textsuperscript{88} were not able to demonstrate 2-PAM iodide in the cerebrospinal fluid in a man who received 44 mg. per kilogram of this oxime intravenously. However, rapid returns to consciousness following administration of 2-PAM iodide have been noted in patients poisoned with parathion.\textsuperscript{89,49,121} This clearing of consciousness would seem to be an indication that the drug has access to the brain in man. The practical clinical result may be further evidence for species variation in the action of 2-PAM.

In contrast to the peripherally-acting quaternary oximes (2-PAM and TMB-4), the nonquaternary oximes have a predominantly central action.\textsuperscript{146,19} DAM has been shown to penetrate the blood-brain barrier and effect some reactivation of brain cholinesterase.\textsuperscript{88,43} However, DAM in large doses has produced coma in man.\textsuperscript{87}

O'Leary et al.\textsuperscript{118} have reported that atropine and a combination of a monoquaternary and a bisquaternary oxime were superior to atropine with either single type of oxime in dogs and rabbits poisoned with sarin or tabun. A 1:1 mixture of 2-PAM chloride and TMB-4 dichloride was the most effective combination tested. Edery and Schatzberg-Porath\textsuperscript{44} have noted synergism between 2-PAM and DAM.

At any given dosage with any given inhibitor and reactivator, the extent of reactivation of cholinesterase activity that occurs depends upon how long the inhibitor and enzyme have been in contact.\textsuperscript{76} Hobbiger theorized that the phosphorylation of cholinesterase led initially to an unstable phosphorylated enzyme which gradually changed to an irreversibly phosphorylated enzyme. This phenomenon has been termed "aging" by O'Brien\textsuperscript{116} and has been shown to occur in vivo\textsuperscript{78} as well as in vitro.\textsuperscript{151,92} Aging occurs rather slowly with methylphosphorylated cholinesterase, but more rapidly with diethyl- or diisopropyl-substituted enzyme.\textsuperscript{92} These results indicate that the oximes might be less effective in regenerating cholinesterase inhibited during repeated exposure than that inactivated after a single exposure.

The use in vitro of a reactivator in the routine cholinesterase test has been proposed to allow determination of both inhibited and normal enzyme levels in a single blood sample from a patient in whom poisoning is suspected.\textsuperscript{3} The occurrence of aging would seem to make achievement of this objective unlikely in connection with repeated exposure; but it should be possible, by use of a similar technique, to differentiate between an inhibition of cholinesterase due to a recent, acute exposure and a depression due to a previous or repeated exposure.

Direct reaction between 2-PAM and the organic phosphorus molecule has been shown to occur.\textsuperscript{143,77} The importance of this reaction in either the prevention of dermal absorption or local effects of anticholinesterase compounds or both has been discussed by Summerson.\textsuperscript{159} Kewitz, Wilson, and Nachmansohn\textsuperscript{93} have, however, pointed out that it is unlikely that the direct reaction is an important factor in the antidotal action on systemic effects in vivo, since in vitro experiments have shown that this direct reaction is very slow at concentrations obtainable under physiological conditions. These authors note that even with a $10^{-8}$M. concentration of 2-PAM iodide at 25 C, pH 7.8, only about
1% of diethyl p-nitrophenyl phosphate (Paraoxon, 10^{-4}M.) reacts per hour. Cohen and Wiersinga found that both DAM and MINA detoxified sarin during in vitro incubation. No detoxication of sarin by 2-PAM iodide occurred. These authors suggested that DAM acts mainly by virtue of direct reaction with the toxicant, that 2-PAM acts primarily by reactivating inhibited enzyme, and that MINA shows both types of activity.

At sufficiently high dosages, 2-PAM iodide is capable of inhibiting both serum and erythrocyte cholinesterase in vitro. Although the antidotal dosages which have generally been used are below the toxic level, there would seem to be, on the basis of studies reported by Loomis, a definite possibility of lessened antidotal effect if greater than the optimal dose of 2-PAM were given. This author noted that dogs given an intravenous dose of 60ug. of sarin per kilogram required more than 240 minutes to recover their normal response to injected acetylcholine. Following intravenous administration of 10, 25, or 250 mg. per kilogram of 2-PAM iodide to normal dogs, <2, 3, and >120 minutes, respectively, were required for return of the normal acetylcholine response. The same dosage levels of 2-PAM iodide when given to sarin-poisoned dogs, produced return to normal acetylcholine response after 40 to 45, 80 to 100, and >120 minutes, respectively. These results indicate that 2-PAM iodide is a cholinesterase inhibitor, that 10 mg. per kilogram was the optimum antidotal dosage under the condition of the test and that larger doses were less effective.

Woodard gave 50 mg. per kilogram of 2-PAM iodide to cattle intraperitoneally and intravenously. This dosage produced some restlessness and mild abdominal distress, but the signs were mild and of short duration. One sheep given 25 mg. per kilogram of 2-PAM iodide intravenously showed the same signs as the cattle, plus an increased respiratory rate and excessive salivation. No change in erythrocyte cholinesterase level was observed in any of these animals.

The cholinesterase inhibitory activity of 2-PAM has also been noted by Bergner and Wagley. These workers reported that 2-PAM iodide in high concentration in vitro had a dual effect on motor end-plate cholinesterase previously inactivated by TEPP. The 2-PAM iodide reactivated the enzyme and, if allowed to remain in contact with the preparation, inhibited the reactivated enzyme.

It is important to note that in poisoned people given therapeutic doses, the inhibition of cholinesterase by 2-PAM is trivial, while the release of enzyme inhibited by the organic phosphorus compound is highly significant.

Kewitz et al. have carried out experiments which show that 2-PAM does not have an atropine-like action. In studies on the blood pressure of eviscerated cats, the effect of acetylcholine was not modified by 2-PAM iodide, even when large doses were used; this effect was completely abolished by a small dose of atropine, however. After 100 mg. per kilogram of 2-PAM iodide, these authors reported a rise in blood pressure from 120 to 160 mm. Hg which lasted 15 to 20 minutes and was abolished by atropine. This effect from the very large dose of 2-PAM iodide was not further studied. Possibly, it was a reflection of the cholinesterase inhibitory effect of 2-PAM.

Both 2-PAM iodide and DAM, when injected intravenously in large doses, have a direct depressant action on the respiratory center. The duration of action of a single dose of 2-PAM has been studied by Kewitz et al. Following intraperitoneal dosage, 2-PAM iodide reached its maximum effect in rats within 30 minutes and had declined to about 50% of maximum effectiveness at the end of the first hour. A further decline in activity took place during the second hour, but even at the end of 2 hours, some protective effect was still evident. Following intravenous dosing, 2-PAM iodide appears to be well dispersed in all body tissues with the exception of the brain. These same authors found that 2-PAM iodide was rapidly excreted in man following intravenous dosage. The half-life was 0.9 hours. Excretion was primarily by the kidneys. In nephrectomized dogs and rats and in a patient with chronic nephritis
and azotemia, excretion was retarded. In vitro, 2-PAM iodide was metabolized aerobically by rat liver slices.

P2S is also rapidly excreted in man following intravenous dosing. Absorption of P2S after intramuscular or oral administration was not satisfactory.

DAM is excreted somewhat more slowly in man than is 2-PAM iodide. The half-life for the former compound is 7.2 hours. Relatively little of the DAM could be recovered from the urine. Apparently, the liver is of major importance in the metabolism of DAM.

Loomis has tried 2-PAM in the treatment of local ocular effects of TEPP and sarin in rabbits. He found that 2-PAM iodide applied locally was very effective in reversing miosis induced by organic phosphorus compounds. The reversal was brought about by 2-PAM iodide in 2 to 3 hours; whereas, if allowed to remit spontaneously, it required from 24 to 48 hours or even longer. The most effective dosage form was a petrolatum-based ointment, containing 0.1% of 2-PAM iodide. In contrast to Loomis' results, Kewitz et al. reported that local application of 2-PAM iodide had no effect on a rabbit's pupil constricted by placing diethyl p-nitrophenyl phosphate in the eye. However, intravenous injection of 50 mg per kilogram of 2-PAM iodide brought the pupil back to normal size and restored reactivity to light within 5 minutes. The formulation in which 2-PAM iodide was used for local application was not stated. It may be that the lack of effect was due to use of a water solution of 2-PAM iodide rather than an oil-based preparation.

Application of 2-PAM iodide and MINA relieves the neuromuscular block caused by anticholinesterases. Grob and Johns reported that DAM reversed the local neuromuscular block produced by anticholinesterases in man, but Edery did not note this effect in the cat or the rat. In this connection, it would be of interest to study the effect of these oximes on the paralysis caused by certain organophosphorus compounds. Holmes and Robins have stated that the reversal of neuromuscular block is due entirely to a reactivation of inhibited cholinesterase in the muscle. Wislicki noted that DAM reduced direct and indirect muscular excitability to a marked extent, while with 2-PAM iodide these effects were slight and were obtained only after intrarterial administration. Application of 2-PAM antagonizes the block caused by the competitive type of blocking agent, such as curare, and it intensifies the effect of suxamethonium. DAM has no definitive influence on the former but prolongs the action of the latter. Neostigmine, unlike atropine, antagonizes the depression of muscle excitability by DAM but has no marked effect on the action of 2-PAM.

Longo et al. have reported that the threshold level of sarin necessary to produce a "grand mal" electroencephalographic pattern was increased about threefold by 2-PAM iodide. However, 2-PAM iodide had no effect on the early phase of desynchronization of the electroencephalogram.

**Antidotal Efficacy of Oximes in Poisoned Experimental Animals**

There are now in the published literature numerous reports giving results of the usage of 2-PAM salts and other oximes in laboratory animals poisoned with various organic phosphorus compounds. The compounds studied have included demeton, DFP, Diazinon, dimefox, dimethoate, endothion, Ethyl Guthion, Guthion, methyl demeton, methyl parathion, morphothion, paraoxon, parathion, phencapton, phosdrin, phosphamidon, Phospholine Iodide, safin, tabun, TEPP, and phorate. Most workers have found that the combination of oxime and atropine is superior to either antidote used alone. However, Sanderson did not note any increased benefit of an oxime (2-PAM iodide or P2S) plus atropine in comparison with atropine alone in rats given oral doses of 10 organic phosphorus and carbamate in-

† References 102, 6, 147, 155, 35, 145, and 52.
secticides. With oral Guthion, Ethyl Guthion, demeton, and morphothion, 2-PAM iodide apparently reduced the beneficial effect of atropine. This author did note potentiation between atropine and oxime in some instances when the poison was given intraperitoneally. Sanderson and Edson concluded that the relative effectiveness of oxime therapy with different organic phosphorus insecticides depends on the proportion of reversible cholinesterase inhibition present, as predicted on the basis of the structure and duration of action of the anticholinesterase compound.

The efficacy of 2-PAM in the treatment of poisoning varies from compound to compound, but it has shown some effectiveness in the case of all the materials listed above with the exception of dimefox, dimethoate, and OMPA. In this connection, it is interesting to note that Tammelin and Enander reported synthesis of an organic phosphorus compound (cholinyl methylphosphonofluoridate) which caused an inhibition of cholinesterase that could not be reversed in vitro by 2-PAM iodide.

Woodard has reported results of studies with 2-PAM iodide in cattle and sheep poisoned with organic phosphorus insecticides. Neither atropine nor 2-PAM iodide alone was a satisfactory antidote in these animals. A combination of 2-PAM iodide and atropine gave good results on cattle poisoned with parathion or Diazinon, but not on cattle poisoned with malathion or on sheep poisoned with parathion.

It appears likely that different oximes have some specificity not only for different organic phosphorus compounds but also for different species.

The effectiveness of oximes as antidotes has also been tested in poisoning by certain other cholinesterase inhibitors which are not organic phosphorus compounds. The use of 2-PAM iodide has been reported to be ineffective, if not detrimental, in the treatment of poisoning by the carbamate insecticide Sevin in rats and dogs. On the other hand, poisoning in the rat by the carbamate insecticides Isolan and Dimetilan was benefited by 2-PAM iodide. It has also been shown that 2-PAM iodide and DAM reverse the physiological effects of neostigmine (which is a substituted carbamate) and several of its derivatives.

Application of 2-PAM in Poisoning in Man

The oxime 2-PAM has now been used in the treatment of poisoning in man in a number of instances.

Namba and Hiraki have reported a series of 5 cases. All of these cases were classified by the authors as serious—indicating a potentially fatal outcome—if untreated. However, one of those patients was already asymptomatic at the time he received 2-PAM iodide. A second patient who had previously received 10 mg. of atropine showed no clinical improvement immediately after receiving 100 mg. of 2-PAM iodide intravenously. He did, however, improve about 3 hours later (6 hours after onset of symptoms). A third case was given ten 100-mg. intravenous doses of 2-PAM iodide during a period of 3.5 hours. By the end of this time, all symptoms had disappeared. A fourth patient became symptom-free after doses of 500 and 400 mg. of 2-PAM iodide, separated by 10 minutes. The fifth case received 1 gm. of 2-PAM iodide intravenously, which the authors considered to represent the ideal treatment. Within 21 minutes, all the symptoms in this fifth case, including muscle fasciculations, had disappeared.

In 3 of the 5 cases cited above, the erythrocyte cholinesterase level returned to normal in 10 minutes, 40 minutes, and 3.5 hours, respectively. In the fourth case the recovery of the red-cell enzyme was significantly more rapid than that of the untreated control. In the fifth case, it is not clear from the data presented that the red-cell enzyme level was ever significantly depressed. No immediate effect of 2-PAM iodide on plasma cholinesterase was evident, but in all treated cases studied, this enzyme returned to a normal level more rapidly than did the untreated control.

It should be especially noted that the recovery in 4 out of 5 of these human cases was
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after 2-PAM iodide alone. Only 1 of the patients received a combination of 2-PAM iodide and atropine. The work with experimental animals cited above would lead one to expect that a combination of both drugs would be more effective than either alone.

In a later paper, Namba \(^{112}\) reported the results of the use of 2-PAM iodide in an additional 26 cases of accidental parathion poisoning and in 2 cases of attempted suicide by ingestion of parathion. The accidental poisonings were treated with 1 to 2 gm. of 2-PAM iodide intravenously. Marked improvement was noted within 30 minutes. Pallor was the only symptom remaining in 5 cases, miosis in 3 cases, and difficulty of speech and tachypnea in 2 cases. These remaining symptoms had cleared within 1 hour after treatment. Complete recovery of consciousness occurred in 5 cases. Symptoms such as nausea, headache, dizziness, and numbness of extremities did not respond as well to 2-PAM as did the others just mentioned. Some of these effects persisted for as long as 1 week. To be effective in cases of ingestion of parathion, it was necessary to give very large doses of 2-PAM. Continuous intravenous infusion of 2-PAM at the rate of 0.5 gm. per hour is preferred by Dr. Namba. One patient received a total dose of 40.5 gm. of 2-PAM iodide, recovering without apparent ill effect. Namba \(^{112}\) mentioned exploratory study of the use of 2-PAM for the prophylaxis of parathion poisoning.

Funckes \(^{62}\) has reported the successful treatment with 2-PAM iodide and atropine of a severe case of occupational parathion poisoning. This patient was given 1 gm. of 2-PAM iodide intravenously about 1 hour after illness began, and he improved dramatically within 10 minutes. Although blood cholinesterase levels prior to administration of 2-PAM iodide were not determined, the enzyme activities after the drug was given indicated reactivation of erythrocyte cholinesterase (0.32 ΔpH per hour) as measured by the method of Michel \(^{107}\) and expressed in terms of change of pH per hour; there was little or no effect on plasma enzyme (0.04 ΔpH per hour).

Quinby and Clappison \(^{121}\) used 2-PAM iodide in the treatment of a 2-year-old child who ate dirt contaminated with parathion. The child was given an intravenous dose of 250 mg. of 2-PAM iodide following repeated doses of atropine. The critically ill, unconscious child returned to an almost normal state in less than 20 minutes after the oxime dosage was given. The levels of both plasma and erythrocyte cholinesterase were near 0 before 2-PAM, but 20 minutes after its administration, the erythrocyte cholinesterase level had returned to within the range of normal. The reactivation of plasma enzyme level was much slower, the normal level being reached between the third and nineteenth days. p-Nitrophenol was excreted in the urine quite rapidly, clearing in about 30 hours from ingestion.

Schwartzman and his colleagues \(^{132}\) treated a child who had ingested a commercial solution of parathion. The treatment included milk, atropine, stropanthin, and 300 mg. of 2-PAM sulfonate. The child regained consciousness and began to improve after treatment; he was discharged from the hospital after 3 days.

Karlog et al. \(^{89}\) have reported the treatment with atropine and 2-PAM iodide of a patient who attempted suicide by taking an estimated 1.75 gm. of parathion by mouth. This patient was given very large doses of atropine beginning soon after poisoning. His respiration ceased, and he was given artificial respiration for 5 hours after poisoning. He was not given any 2-PAM until 2.5 days after poisoning. During the next 12 hours he was given 3.5 gm. of the antidote. Each dose of 2-PAM iodide produced transitory rises in plasma and erythrocyte cholinesterase activity which persisted for only a few hours, but the patient eventually recovered.

Rosen \(^{125}\) treated with 2-PAM iodide a young man who was poisoned by accidentally spilling a gallon bottle of parathion concentrate on his hands. This patient was given adequate doses of atropine during the first 18 hours after poisoning, and by the end of this time he was symptom-free except for per-
sisting muscular weakness. After 2-PAM was administered, prompt improvement in muscle strength was noted.

One of the most severe poisoning cases treated with 2-PAM is that reported by Imo. A young girl drank parathion with suicidal intent. She was found unconscious, in convulsions, foaming at the mouth, and unreactive even to the strongest stimulus. She had almost no pulse and was cyanotic and areflectic. Atropine, artificial respiration, Stereofundin, and nikethamide (Coramine) were given, and spontaneous respiration was restored. About 9 hours later, 500 mg. of 2-PAM iodide was given. Within 1 hour, the patient regained color, became completely conscious, and her pupillary reflexes returned to normal. Further recovery was uneventful.

Other instances of parathion poisoning treated with 2-PAM include a series of 10 cases (of which 6 were attempted suicide) reported by Erdmann. Three of these patients recovered, 3 died, and in 4 cases the outcome is not indicated.

Erdmann and Latki have used 2-PAM iodide in the treatment of a case of poisoning from inhalation of DFP. The administration of atropine and 2-PAM iodide (2 doses of 500 mg. each, orally, 2 and 5 hours after exposure) produced prompt remission of symptoms with the exception of miosis, which persisted for 8 to 10 days and led to a severe conjunctivitis. Some increase in erythrocyte cholinesterase activity was noted after 2-PAM, but the enzyme level did not return to normal until about 30 days later.

In 2 cases of moderately severe 2-carbomethoxy-1-methylvinylidimethyl phosphate (Phosdrin) poisoning, Funckes noted a less dramatic clinical response to 2-PAM iodide than in the case of parathion poisoning. Both of the 2-PAM-treated Phosdrin cases continued to show symptoms of poisoning for several hours after receiving the antidote. However, the erythrocyte cholinesterase level was significantly increased, and the plasma enzyme was regenerated to a lesser degree after 2-PAM therapy.

A formulating plant worker studied by Quinby and Congdon became poisoned while working with parathion. He had a very low blood cholinesterase level (plasma 0.10 and erythrocytes 0.08 \( \Delta \) pH per hour) during his acute illness and was given atropine therapy. His gastrointestinal complaints, particularly anorexia and nausea, as well as weakness, headache, and malaise, persisted. He continued to work in the plant, although presumably he was removed from direct contact with insecticides. However, his urinary \( p \)-nitrophenol excretion indicated continued exposure to parathion. Because of these continuing symptoms, and in an attempt to raise his blood cholinesterase level sufficiently to permit his safe return to his regular work, this man was given 1 gm. of 2-PAM chloride 6 days after his acute illness. The antidote had little or no effect on his blood cholinesterase level, but he felt much better the next morning.

It is apparent from brief case reports that the use of 2-PAM in combination with atropine is considered effective and is routine in the treatment of poisoning by organic phosphorus insecticides in Israel.

Suggestions for Treatment

The following suggestions are provided as a guide to the physician who may be called upon to treat persons poisoned by anticholinesterase compounds:

I. In very severe cases, the order of treatment should be as follows:

1. ARTIFICIAL RESPIRATION, preferably by mechanical means.
2. ATROPINE, 2 to 4 mg. (1/30 to 1/15 grain) intravenously as soon as cyanosis is overcome. Repeat at 5- to 10-minute intervals until signs of atropinization appear (dry, flushed skin and tachycardia as high as 140 per minute).
3. 2-PAM, 1 gm. slowly, intravenously.
4. DECONTAMINATION of the skin, stomach, and eyes as indicated.
5. SYMPTOMATIC TREATMENT.

II. In the more usual case, proceed as follows:

1. ATROPINE, 1 to 2 mg. (1/60 to 1/30 grain), if symptoms appear. If excessive secretions occur, keep the patient fully atropinized. Give atropine sulfate every hour up to 25 to 50 mg. in a day.

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(2) DECONTAMINATION of the skin, stomach, and eyes as indicated.

(3) 2-PAM, 1 gm. slowly, intravenously if the patient fails to respond satisfactorily to atropine.

(4) SYMPTOMATIC TREATMENT.

It will be noted that this recommended dosage of atropine is greater than that conventionally employed for other purposes but is within safe limits. People poisoned by anticholinesterase organic phosphorus compounds have an increased tolerance for atropine. For additional discussion of atropine usage, see "Use of Atropine and Other Nonspecific Antidotes," above.

Although different oximes offer somewhat different therapeutic possibilities, 2-PAM appears to be the most generally useful oxime now available. There appears to be no essential difference in the effects of the different salts of 2-PAM, and 2-PAM chloride does have the advantage of greater solubility and lack of iodine taste as compared to 2-PAM iodide, which used to be more easily available. A dosage of 10 to 20 mg. per kilogram has proved a safe but generally effective level. As a round figure, 1 gm. should be an average adult dose. Intravenous administration is recommended to obtain rapidity of action. Since 2-PAM is excreted rapidly, repeated doses may be necessary, particularly in cases involving parathion or other compounds that must undergo bioactivation before becoming toxic. It is important to note, however, that the only known signs or symptoms of overdosage with 2-PAM are identical to the manifestations of poisoning with anticholinesterases. 2-PAM should be administered in conjunction with adequate doses of atropine. Other supportive treatment, including particularly artificial respiration, should also be carried out as required.

The value of 2-PAM is most thoroughly established in connection with the treatment of poisoning by parathion. When used in cases of poisoning by other organic phosphorus insecticides, it should be with the awareness that it may be somewhat less effective. In fact, 2-PAM appears to be ineffective in treating poisoning due to OMPA. Until 2-PAM is freely available for prescription it is recommended that the limited supply not be drawn upon in cases of mild poisoning or in cases that show a completely satisfactory response to atropine.

Consideration should be given to local application of 2-PAM to the skin to reduce dermal absorption in exposed persons, and to the eyes to reverse organic-phosphorus-induced miosis. The use of 2-PAM for the prevention rather than the treatment of systemic poisoning has been proposed. These procedures seem to be safe on the basis of limited studies with experimental animals, although their effectiveness has not been proved. These procedures are strictly exploratory, while the value of 2-PAM for treatment of systemic poisoning, especially by parathion, is established.

Never give morphine, theophylline, or theophylline-ethylene-diamine (aminophylline) to a patient poisoned with an anticholinesterase agent. Do not give atropine to a cyanotic patient; give artificial respiration first, and then give atropine. Large amounts of intravenous fluids are generally contraindicated because of excessive fluid in the respiratory tract. Tranquilizers should be used with great caution; they are seldom indicated at all. Succinyl choline should not be given.

If pulmonary secretions have accumulated before atropine has become effective they should be removed by suction and a catheter. If the stomach is distended, empty it with a Levine tube.

If the patient has not yet shown symptoms or they have been allayed by treatment, he must be completely and quickly decontaminated. Remove the patient's clothing and, with due regard for his condition at the moment, bathe him thoroughly. Remove any visible insecticide gently with a generous amount of soap and water or other detergent if available. Avoid abrasion. When the skin appears clear, bathe or swab with ethyl alcohol. Parathion and many of the other organic phosphorus insecticides are very much more soluble in alcohol than in water, and significant amounts can be washed from skin.
that has been scrubbed several times with soap and water.

If there is any suspicion that the poison has been ingested or inhaled and if the patient is still responsive, induce vomiting, give some neutral material such as milk or water, and induce vomiting again. The reason for mentioning inhaled material is, of course, that a large portion of it is deposited in the upper respiratory tract and subsequently carried to the pharynx and swallowed. Nausea may be anticipated on the basis of the systemic action of these compounds, but if vomiting is not profuse, gastric lavage may be used. Experiments have indicated that vomiting induced immediately or even 1.5 hr. after ingestion is more effective than gastric lavage in removing poison.

It must be kept in mind how little paraquat is necessary to produce poisoning by the oral route. Repeated dosage at the rate of 7.2 mg. per man per day led to moderate reduction of blood cholinesterase activity. A dose of 25 to 50 mg. was fatal to a child, and a dose of 120 mg. was fatal to an adult.

Atropine does not protect against muscular weakness. The usual mechanism of death appears to be respiratory failure. The use of an oxygen tent or even the use of oxygen under slight positive pressure is advisable and should be started early. Watch the patient constantly, since the need of artificial respiration may appear suddenly. Equipment for oxygen therapy and for artificial respiration should be placed at the patient's bedside in readiness while the patient is on his way to the hospital. Cyanosis should be prevented by the most suitable means, since continued anoxia aggravates the depression of the respiratory center caused by the poison directly. Complete recovery may occur even after many hours of artificial respiration have been necessary.

If there is any reason to think that the eyes may have been contaminated, irrigate them with isotonic saline solution or water. The absorption of some of the organic phosphorus insecticides by the eye is remarkably rapid.

The acute emergency lasts 24 to 48 hours, and the patient must be watched continuously during that time. Favorable response to one or more doses of atropine does not guarantee against sudden and fatal relapse. Medication must be continued during the entire emergency. Any person who is ill enough to receive a single dose of atropine should remain under medical observation for 24 hours, because the atropine may produce only a temporary relief of symptoms in what may prove to be a serious case of poisoning. Atropine should never be administered for preventive purposes to persons who have not become sick.

Miosis and headache may persist after recovery from poisoning by organic phosphorus insecticides is otherwise largely complete. In some cases, the systemic administration of atropine is followed by partial or temporary dilatation of the pupils. Miosis responds more dependably to 2-PAM. If further systemic treatment is not necessary, the miosis and associated headache will respond to the instillation of 0.5% to 1% atropine solution or 0.5% atropine ointment into the eyes.

Following exposure heavy enough to produce symptoms, further organic phosphorus insecticide exposure of any sort should be avoided. The patient may remain susceptible to relatively small exposures to the same or any other organic phosphorus compound until regeneration of cholinesterase is nearly complete.

Prevention of Poisoning

The most important single factor in the prevention of poisoning is a knowledge of the hazards involved in handling the various anticholinesterase compounds. It is essential that the facts about the toxicity of these materials be learned through research and then that these facts be made known to all concerned, including the formulating plant worker, the commercial sprayman, the farmer, the householder (who may purchase these insecticides for use in his house or garden and, if not adequately warned, may leave the toxic materials accessible to children or other irresponsible persons), and the
physician who may be called upon to treat poisoning.

In persons occupationally exposed, poisoning can best be prevented by constant, thoughtful care and by use, where indicated, of safety devices such as protective clothing, respirators, masks, air-conditioned cabs, or special factory ventilation.

Prevention can also be approached by observing trends in serial blood cholinesterase determinations on persons constantly exposed. The interpretation of individual values in asymptomatic persons is difficult. It is clear, however, that a single very low enzyme value for one worker or a low average value for a group of exposed workers is an indication of the need for improved personal care or better mechanical protection or both. In a similar way, prevention of poisoning may be helped by proper evaluation of the amount of urinary excretion of biotransformation products.

Direct methods are also available to measure the exposure which spray operators have to pesticides during their usual conditions of work.42

Severe poisoning may at times be prevented by the immediate recognition and correction of contamination. Prompt, effective medical treatment may prevent a mild poisoning case from developing into a serious one.

Comment and Summary

Poisoning due to organic phosphorus compounds may be encountered in relation to their use as insecticides; as chemical warfare agents; or as drugs for the treatment of abdominal distention, glaucoma, or myasthenia gravis. The principal, if not the only, pharmacologic action of the organic phosphorus insecticides is inhibition of the enzyme cholinesterase. The inactivity of this enzyme results in an accumulation of unhydrolyzed acetylcholine and the appearance of signs and symptoms referable to overstimulation of the parasympathetic nervous system. The drug of choice in the treatment of organophosphorus poisoning has been atropine. Atropine therapy produces relief of certain symptoms based on blocking the action of excess acetylcholine, but it has no effect on the basic biochemical pathology involved in anticholinesterase poisoning. With regard to symptoms, atropine is effective in treating those effects referable to the central and muscarinic action of acetylcholine but has no effect on the nicotinic action.

More recently a series of antidotes has been developed that are able to reverse the combination between the cholinesterase molecule and the inhibitor. These compounds are oximes and include salts of, for example, 2-PAM (2-pyridine aldoxime methiodide), DAM (diacetylmonoxime), MINA (monoisonitrosoacetone) and TMB-4 (1,1'-trimethylene bis(4-formyl-pyridinium bromide) dioxime). Since the oximes and atropine have different sites of action in the mammalian body, it is preferable to use these 2 agents in combination and thus take advantage of the antidotal effects at both points.

The most widely used of the oximes in treating poisoning is 2-PAM. This compound has at least 3 actions that may be of importance in the treatment of poisoning by an organic phosphorus compound. These actions are: (1) reactivation of inhibited cholinesterase, (2) reaction with and inactivation of the organic phosphorus molecule, and (3) inhibition of cholinesterase. Atropine has none of these actions. The dominant effect of therapeutic doses of 2-PAM in poisoned animals is reactivation of inhibited cholinesterase. Application of 2-PAM reactivates inhibited cholinesterase both in vitro and in vivo, except that it has little or no effect on the enzyme in the brain. The non-quaternary oximes (such as DAM and MINA) are able to penetrate the blood-brain barrier. With any given inhibitor and reactivator, the extent of reactivation of cholinesterase activity depends upon how long the inhibitor and enzyme have been in contact.

Although the value of 2-PAM in the treatment of organic phosphorus poisoning was discovered in the United States and procedures for its use were developed here, and although this antidote is now widely available for use by physicians in a number of other
countries (England, Israel, Japan, and New Zealand), it can be purchased in this country only by qualified physicians for clinical investigational use. It is unfortunate that no firm has seen fit to market 2-PAM commercially for prescription use in the United States.

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ORGANIC PHOSPHORUS POISONING


Durham—Hayes


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HEALTH HAZARDS OF PESTICIDES

The pesticide-related problem that the average physician is most likely to face and about which he must take immediate and definitive action is acute poisoning due to occupational or accidental exposure or to suicidal ingestion.

Almost every pesticide can cause poisoning if it is swallowed. For many years and at least as late as 1959, the arsenicals caused more accidental deaths than any other group of pesticides. This is almost certainly due to the casualness with which they are still stored and the ease with which they can be found and ingested by children and other uninformed people. The arsenical pesticides have never been an important source of occupational disease. Among the newer pesticides, greatest attention has focused on the chlorinated hydrocarbon and the organic phosphorus insecticides. Although the chlorinated hydrocarbons have caused a moderate number of cases of accidental poisoning, their record of occupational safety is generally good. In fact, the great amount of study they continue to receive is justified almost exclusively by their persistence as residues on treated crops and their relatively prolonged storage in the tissues of domestic animals and man. However, careful regulation has prevented the residues from offering anything but a potential problem. There is no confirmed record of clinical effect from eating food treated with pesticides according to approved agricultural practice.

From the standpoint of occupational hazard, the more toxic organic phosphorus insecticides are the outstanding offenders among the newer agricultural chemicals. This is true in spite of the strict regulation imposed on them from the beginning. This regulation and the extensive educational campaign that has accompanied their introduction and use undoubtedly have restricted both occupational and accidental poisoning by these compounds. Granted that all noncriminal poisoning can be traced to "carelessness," the growing importance of the more toxic organic phosphorus insecticides as reported causes of occupational disease is almost certainly due to the tremendous increase in their use, to their inherent toxicity, and to the ease with which many of them are absorbed through the skin.

Two factors specifically favor success in the treatment of poisoning with the organic phosphorus compounds, i.e., the short duration of the emergency and the existence of effective antidotes. The acute episode lasts only from 24 to 48 hours. Most deaths occur within the first 24 hours, and if a patient survives this long, he usually recovers completely.

The principal pharmacologic action of the organic phosphorus insecticides, if not the only one, is inhibition of the enzyme cholinesterase. This results in an accumulation of unhydrolyzed acetylcholine and, therefore, in signs and symptoms referable to over-stimulation of the parasympathetic nervous system. The drug of choice in the treatment of organophosphorus poisoning has been atropine, which relieves certain symptoms by pharmacologic blocking of the action of acetylcholine. Atropine, however, has no effect on the basic, biochemical pathology involved in anticholinesterase poisoning. Furthermore, it has no effect on the nicotinic action of acetylcholine although it is effective in treating those symptoms referable to the central and muscarinic actions of this neurohumor.

More recently a series of antidotes has been developed that can reverse the combination between the cholinesterase molecule and the inhibitor. These compounds are oximes. The outstanding members of this group are the salts of 2-pyridine aldoxime, including the methiodide (2-PAM iodide), the methochloride (2-PAM chloride), and the ethanesulfonate (P2S). 2-PAM chloride is sold in the United States by the Campbell Pharmaceutical Company, Inc., 121 East 24th St., New York City, under the trade name Protopam. This drug is marketed under a limited license for sale to qualified physicians for clinical investigations only. Emergency supplies are available at some poison control centers, at many medical schools, or from the U.S. Public Health Service laboratories in Atlanta, Ga., Wenatchee, Wash., and Phoenix, Ariz.

In the July issue of the Archives of Environmental Health (p. 21) there is a review by William F. Durham and Wayland J. Hayes, Jr., on the therapy of organic phosphorus poisoning. Special attention is given in this review to compounds that reactivate inhibited cholinesterase and to their proper clinical use in combination with atropine. Extensive animal research on salts of 2-pyridine aldoxime has been completed. Well-documented, clinical use of these compounds in more than 40 cases of parathion poisoning has established their effectiveness and safety for this purpose. More limited experience indicates their value in the treatment of poisoning by some other organic phosphorus compounds.

Detailed information on the clinical use of these drugs is now available to physicians through the above-mentioned review. For maximum usage to be made of the considerable knowledge now available on treatment of anticholinesterase poisoning, it is necessary that one of these oxime salts be made generally available for prescription by physicians in the United States.