FURTHER DEVELOPMENTS IN THE TREATMENT OF POISONING WITH ALKYLPHOSPHATE (ORGANOPHOSPHATE) INSECTICIDES

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In 1958 I reviewed the then existing methods of treatment of alkylphosphate poisoning.[1,2] In this paper, I shall discuss the more recent knowledge we have gained as to the symptoms caused by, and the mechanisms and modes of action of, different alkylphosphate insecticides, as well as the more successful methods of treatment of cases of poisoning. It is a great step forward in the treatment of cases of alkylphosphate poisoning that a reactivator (Toxogonin) of the inactivated ChE (cholinesterase) *which passes the blood-brain barrier,* has been discovered. Not only does Toxogonin pass the blood-brain barrier but, according to reports in the literature, it is also more effective in cases of poisoning with some organophosphates, and is less toxic than P₂AM which does not pass the blood-brain barrier, or does so only to a very limited extent.

SYMPTOMS OF ORGANOPHOSPHATE POISONING

It is clear that the symptoms of poisoning with organophosphates are due not only to inactivation of cholinesterase (ChE), i.e. cholinergic, but that other factors also have

to be considered as will be gathered from the evidence presented below. It appears obvious that alkylphosphates will affect not only ChE but also other enzyme systems. It is therefore not surprising that reactivators (P₂AM, P₂S, DAM, MINA) of ChE inactivated by organophosphates are not 100% effective in all cases of poisoning with these insecticides. Further, the actions of organophosphates depend upon the nature of the phosphate and on the species. They can cause death by virtue of their central or peripheral action, or both.

According to Hegazy[3] and various other investigators, organophosphate compounds interfere not only with ChE but also with the action of many other enzyme systems, hence 'the poisonous action of organic phosphate compounds could not be explained solely as an inhibition of cholinesterases, and neurological disturbances in patients poisoned by organophosphorus compounds were the result of the stimulant action of organic phosphates on sympathetic ganglia and cerebral centres'.

Hegazy[3] described meta-isosystox poisoning in 126 men employed as sprayers of insecticides and in accidentally-exposed patients. He states: 'Serum cholinesterase levels showed an initial fall and then a rise above normal. The fall of cholinesterase was marked in patients who were exposed for relatively long periods but not in patients accidentally exposed for short times. The rise in serum cholinesterase is thought to be a compensatory protective mechanism.

'Correlation between serum cholinesterase levels and symptomatology was absent in some patients but present in the majority. Cholinesterase inhibition may not be the only mechanism by which organic phosphorus compounds cause poisoning.'

Hobbiger[4] states that it is widely believed 'that organophosphates possess other actions in addition to those mediated by cholinesterase inhibition. The results obtained with 1 particular organophosphate are not applicable to other organophosphates which form the same type of phosphorylated enzyme.'

Hegazy[3] refers to observations made by Hayes and others that 'some degree of tolerance may develop in persons exposed to organic phosphorus compounds for long periods'.

Studt and Wetzel[5] report 'a highly unusual toxic picture with ulcerative stomatitis' in a suicide attempt with parathion. They state: 'This lesion has so far been almost exclusively seen in fatal cases, but the patient in question recovered. The manifestations are ascribed to "endogenous acetylcholine poisoning" with "total autonomic derangement" combined with a toxic-allergic process in the form of a symptomatic thrombopenia.'

Enders and Grupp[6] found Heinz bodies in the red blood cells of rats chronically poisoned with parathion; the blood picture resembled that seen in nitrobenzol poisoning.

According to Hegazy,[3] serum ChE levels are low in cases of malnutrition, liver disease and bilharziasis. Fluctuations in serum ChE levels in the same individual may amount to 10 - 15%. The above phenomena will render the individuals concerned more susceptible to organophosphate poisoning and also stress the importance of the determination of serum ChE levels in all individuals who are likely to be exposed to organophosphates. Special consideration

should be accorded individuals whose employment will expose them to the effects of organophosphates.

Potentiation of the Toxicity of Insecticidal Organophosphates

An article published by Dubois[7] on potentiation of the toxic effects of insecticidal organophosphates among themselves and by other insecticides, by chemicals used in food processing and accidentally contaminating food and drink, and by drugs, is deserving of serious consideration and extensive investigation in the interest of human and animal health. There is ample evidence that various chemicals (pesticides, chemicals used in food processing, and drugs) may either have a potentiation or additive effect or may, to a certain degree, counteract each other's harmful effects on man and animal. The potentiation and/or additive effects of chemical substances may be:

(a) the result of their synergistic pharmacologic or toxicologic actions; or

(b) may be due to the fact that the most important organs of excretion (liver, kidneys) are damaged resulting in delayed excretion of the poison(s) concerned; or

(c) may be due to the chemicals concerned inhibiting or preventing each other's detoxification in the body; or

(d) they may interact chemically in the body forming compounds more (or less) toxic than the original compounds.

An important point in the problem of potentiation or additive effects of chemicals is the *relative quantities* which enter the body.

Minimal quantities of pesticides and/or other chemicals present in articles of food may not potentiate each other's harmful effects or have additive effects, while the ingestion of larger quantities of these chemicals (subacute and acute poisoning) may do so.

Distribution of Parathion in the Body of Exposed Individuals

There is ample evidence that the concentrations of parathion detectable in the various organs of victims of parathion poisoning vary greatly.

In a fatal case of parathion poisoning in a 27-year-old blacksmith, Schweitzer[8] reports that the brain contained 2½ times the amount of parathion that was found in the liver (per unit of weight).

Karlog and Poulsen[9] studied the spontaneous and pralidoxime (P₂AM)-induced reactivation of brain ChE in the chicken after fatal nitrostigmine (parathion) poisoning. They found that after administration of parathion, brain ChE activity was reduced to 10% immediately after death. Two further points of importance emerged from the above study made by Karlog and Poulsen, namely:

(i) after 8 days of storage normal controls showed a decrease of 25% in ChE activity; and

(ii) during postmortem storage a gradual reactivation of the inactivated ChE occurred up to the extent of 50% of the normal. These findings are of great importance as far as the collection of specimens for ChE determinations is concerned.

TREATMENT OF CASES OF ORGANOPHOSPHATE POISONING

The curative effects of mono-*iso*nitrosoacetone (MINA) and of diacetyl monoxime (DAM), pyridine aldoxime methiodide (P$_2$AM) and other oximes in cases of organophosphate poisoning have been investigated.

Investigations conducted by Askew et al.[10] have shown that the therapeutic properties of the various oximes differ in degree in their curative effects in cases of poisoning with different organophosphates. These authors compared the chemical, biochemical and therapeutic properties of MINA, DAM and P$_2$AM and found a number of interesting anomalies. They state: 'Thus, their relative therapeutic potencies are not entirely consistent with their reactivities with organophosphates or with their reactivating powers. Whereas MINA and P$_2$AM react at about the same rate with organophosphates, DAM reacts more slowly, while the reactivation powers fall in the order P$_2$AM > MINA > DAM; yet DAM and MINA are equally effective in animals poisoned with *iso*propyl methylphosphonofluoridate (Savin) but P$_2$AM is ineffective.'

Rosenberg and Coon[11] found that in rats poisoned with EPN (ethyl-p-nitrophenyl-thionobenzene phosphonate) mortality was reduced by the administration of nikethamide and nicotinamide. They suggest that this beneficial effect was due to the protection of tissue cholinesterase. This investigation by Rosenberg and Coon was prompted by findings reported by DiStefano and his co-workers, namely, that 'intraperitoneal injection of nikethamide plus atropine in female rats increased the oral LD$_{50}$ of simultaneously administered ethyl-p-nitrophenyl thionobenzene phosphonate (EPN) approximately 10 times, whereas atropine alone only doubled the LD$_{50}$'.

In the course of a clinical evaluation of 24 cases of organophosphate poisoning treated with atropine and pralidoxime, Quinby[12] found that the use of pralidoxime (= 2-pyridine aldoxime methyliodide and 2-pyridine aldoxime methylchloride) was most valuable in parathion poisoning, but less so in cases of phosdrin poisoning. Too few cases of tetraethyl pyrophosphate and malathion poisoning were treated to allow reliable clinical evaluation; however, the results were promising. The side-effects induced by pralidoxime were minimal.[12]

Pralidoxime iodide may induce one or more of the following symptoms: dizziness, blurred vision, diplopia, headache, impaired accommodation, nausea, and tachycardia.

According to Erdmann and Von Clarmann,[13] P$_2$AM does not pass the blood-brain barrier, with the result that the cholinesterases in the brain remain inactivated *for days and even for weeks*. According to a note prepared by a medical panel,[14] 'The rate of recovery of cholinesterase activity depends on the compound which was responsible for the poisoning and may take 2 or 3 months'. Many authors have expressed the view that prolonged inactivation of the brain cholinesterases have been responsible for the psychic disturbances (paranoid and depressive reactions) exhibited by the patients concerned.

It was therefore most desirable to find an antidote to organophosphate poisoning which will pass the blood-brain barrier, thus reactivating the blocked cholinesterases.

Erdmann and Von Clarmann[13] found that in cases of E605 (parathion) poisoning, Toxogonin [LüH6, dichloride of bis-(4-hydroxyiminomethyl-pyridinium-(1)-methyl)-ether] in doses of 250 mg. intravenously, (i) acts *quicker* and *better* than P$_2$AM, and (ii) is quite safe when administered to normal individuals in doses which are effective in cases of E605 poisoning.

It is stated that Toxogonin passes the blood-brain barrier and is a more active antidote as it is effective in smaller doses than P$_2$AM.

Views as to the distribution of pyridoxine-2-aldoxime methiodide (P$_2$AM) in the different organs and systems of the human and animal bodies differ, and this fact prompted Loomis[15] to conduct further investigations. He studied the distribution and excretion of methyl-^{14}C tagged P$_2$AM in mice and dogs. The results of this investigation showed that 'after intravenous injection, P$_2$AM penetrates readily into the liver and kidney of mice and into most soft tissues of the dog. No isotope was found in the brain of the mice, and only trace amounts of isotope were found in the brain of 1 dog. Isotope is retained in the tissues except for the brain after it has been cleared from the blood'.

Loomis confirmed the finding that the combined use of atropine and oxime treatment in anticholinesterase poisoning yields the best results. The explanation is that in this case atropine protects the central nervous system from effects of the antisterase.

Erdmann et al.[16] conducted tests on 12 male volunteers (20-28 years old) and found that intramuscular injection of Toxogonin was painless. The test subjects testified that 5-10 minutes after injection of Toxogonin they experienced (a) a warm and tense feeling in the face muscles and a decrease in movement in the mimic face muscles; (b) a cold sensation, on inspiration, in the nose-palatal region (like that caused by menthol). All these symptoms disappeared 2-3 hours after the injection.

Each of the test individuals received 250 mg. of Toxogonin intramuscularly. Twenty to thirty minutes after the injection a maximum blood level of approximately 6 μg./ml. was recorded. Six hours after the injection the blood level of Toxogonin had dropped to 1-2 μg./ml. Erdmann et al.[16] state that Toxogonin is approximately 3 times more active than P$_2$AM in organophosphate poisoning. The dose is approximately 3.0 mg./kg. body-weight intramuscularly.

Kuga and Erdmann[17] investigated the action of Paraoxon on the centres of respiration and of the vagus and the effect of various antidotes. They found that 'the peripherally-acting antidotes Pralidoxime or methylatropine have no protective or therapeutic effects on the spastic paralysis of the respiratory movements of the glottis. An excellent antidotal action however was obtained with the central-acting substances Toxogonin or atropine.'

Heilbron and Tolagen[18] tested the reactivating effects of P$_2$S, TMB-4 and Toxogonin [LüH6, BH6 = bis (4-hydroxymi-nomethyl-pyridinium (1)-methyl)-ether dichloride] on atropinized mice in experimental Savin, Soman and Tabun poisoning and found that of these 3 compounds Toxogonin has by far the most beneficial effects.

DISCUSSION

The following points are of importance as far as the toxicology and treatment of poisoning with insecticidal organophosphates are concerned:

1. Organophosphates do not only inhibit ChE but also other enzyme systems, with the result that the symptom-complex caused by them is not only cholinergic in nature but covers a much wider range of symptoms.

2. Some cases of organophosphate poisoning may initially show a drop in ChE serum levels and then a transitory rise above normal. This is probably a compensatory protective mechanism.

   This phenomenon should be kept in mind when taking specimens of blood for ChE determinations. Specimens from the same patient should be taken at regular intervals.

3. Correlation between serum ChE levels and symptomatology is absent in some patients, consequently the symptom complex may vary greatly in cases of poisoning with organophosphates.

4. Blood serum ChE levels may be low in cases of malnutrition, liver disease and bilharziasis. Also, in the same individual serum ChE levels may from time to time fluctuate appreciably (10-15%). These 2 phenomena should lead us to execute not only 1 but repeated determinations of serum ChE levels at specified intervals over specified periods before deciding on 'normal levels'. This procedure is of the utmost importance in all cases where individuals may, or will, be exposed to organophosphates (appliers of insecticides, industrial workers, etc.). In all cases, serum ChE determinations should be made before employment.

5. It is important to note that parathion, and possibly also other organophosphates, pass the blood-brain barrier, with the result that they accumulate in the brain, thus reducing ChE levels to extremely low and dangerous levels. What is of still greater importance is that the valuable antidote P₂AM apparently does not pass the blood-brain barrier, thus permitting the toxic organophosphates to exert their harmful effects on the central nervous system *for extended periods*. The ChE in the brain may be, and often is, inactivated to a serious degree (up to 90%) and may remain blocked for days and even weeks or months. The prolonged inactivation of brain ChE is probably responsible for the mental confusion and psychic disturbances often exhibited by patients suffering from organophosphate poisoning.

6. The same oxime varies in its capacity as a curative agent in cases of poisoning with different organophosphates; hence the importance of (a) establishing the most effective and safest antidote in the case of each organophosphate; and (b) the screening of existing and any newly-developed oximes in order to establish which are the most effective and safest and which of them has the broadest spectrum of activity.

7. In comparison with P₂AM, Toxogonin, according to the available literature, possesses 3 important advantages as an antidote in organophosphate poisoning, namely:
   (a) it passes the blood-brain barrier thus reactivating the seriously inactivated ChE in the brain;
   (b) it acts more quickly; and
   (c) it is safer and more effective.

8. Vandekar et al.[19] assessed the hazards of acute poisoning by carbamates and organophosphates. They found that carbamates are safer than organophosphates as far as fatalities are concerned, and state that the early warning symptoms in cases of carbamate poisoning serve as a means of preventing deaths.

The whole trend in the development of safer pesticides should be to determine (a) the safest (least toxic) and most effective chemical in a given pest; and (b) to discard the most toxic pesticides in favour of other effective and safer chemicals. If these lines of action were adopted, we would perhaps succeed in discovering and using the least toxic organophosphates. In addition it may be possible to replace some of the most toxic organophosphates by less toxic and less dangerous carbamates.

In assessing the toxicity and dangers of pesticides, their relative tendencies to accumulate and persist in the body or cause serious irreversible damage to organs or systems should receive serious consideration. Those pesticides which are inclined to accumulate or concentrate in the central nervous system (brain) should receive special attention. Such poisons are parathion (and possibly other organophosphates) and DDT (and possibly other chlorinated hydrocarbons). These poisons affect certain important enzyme systems detrimentally and their presence and persistence in the brain cannot but be at least partially responsible for the mental confusion and psychotic disturbances exhibited by the patients concerned.

TREATMENT

It should be stressed that the first ¼ hour after organophosphate poisoning is of the utmost importance to the fate of the patient, and the treatment suggested below should therefore be instituted immediately, and in the order stated.

A blood sample should be taken before administration of a cholinesterase activator.

1. Immediate removal of the patient(s) from the source of poisoning.

2. Immediate removal of contaminated clothing. Exposed areas of the skin should be thoroughly washed with soap and water and 3% sodium bicarbonate in water. If the eyes are affected, they should be treated with a 3% sodium bicarbonate solution in water and with analgesic drops for the pain.

3. Atropine sulphate should be injected at the earliest possible moment and *before* a specific cholinesterase reactivator is given.

Von Clarmann[20] suggested the following treatment with atropine: In very serious cases of organophosphate poisoning 2 - 5 mg. should be administered by slow intravenous injection (children 0·3 - 3·0 mg.). If necessary, repeat the injections every 5 - 15 minutes until symptoms of atropinization (mydriasis, dry mouth) appear. In less serious cases, smaller doses of atropine could be given intramuscularly or subcutaneously. In extremely serious cases a total dose of 40 - 100 mg. of atropine sulphate and more was administered within 24 - 48 hours.

It should be kept in mind that atropine competes with acetylcholine and is effective only in treating those effects referable to the central and muscarinic action of acetylcholine, but does not affect the nicotinic action.

Warning: Atropine should be used with the utmost care in cyanotic patients.

Taylor et al.[21] recommend that 10 mg. of *metaraminol* (*Aramine* = *m*-hydroxyphenyl-1-amino-2 propanol-1-[+]-bitartrate) be given parenterally with atropine because:
   (a) it has been shown that the side-effects of atropine will be reduced or obviated and
   (b) 'there is suggestive evidence that metaraminol enhances the antagonistic action of atropine against acetylcholine accumulation'. *Metaraminol* is a long-acting sympathomimetic pressor drug.

4. Reactivators of the inactivated (phosphorylated) cholinesterase. According to our present knowledge concerning the mode of action and distribution of reactivators in the body, it would appear that the safest, most quickacting and most effective reactivator is Toxogonin. Its great advantage over P₂AM is that it passes the bloodbrain barrier.

Unfortunately, our knowledge and experience with Toxogonin is at present very limited and it is suggested that extensive investigations be conducted into its therapeutic action in all cases of poisoning with organophosphates.

Von Clarmann[20] suggests 250 mg. of Toxogonin intravenously. As it is fairly quickly eliminated, administration should be repeated after 1 - 2 hours. Children should receive 4 - 8 mg./kg. body-weight as a single dose intravenously.

At present the most widely used activator is P₂AM, commencing, in serious cases, with 1 G intravenously. Unfortunately, it does not pass the blood-brain barrier, and consequently it has no, or only a negligible, effect on inactivated brain ChE.

5. Gastric lavage should be carried out if the poison had been taken by mouth. Small quantities of fluid should be used repeatedly as large quantities of fluid wash the poison through the pyloric sphincter into the intestinal canal. In order to facilitate hydrolysis and detoxification of the poison an aqueous solution of 0·5 - 1% sodium bicarbonate could be used.

Von Clarmann[20] advises stomach lavage with *carbo medicinalis* as it adsorbs large amounts of organophosphates.

Symptomatic Treatment

(a) *Respiration.* This must be carefully watched and the patient kept under constant supervision. If necessary, artificial respiration should be instituted. Excessive mucus secretion must be removed by endotracheal intubation or even tracheotomy, if necessary. The patient must be kept in a lateral position.

Oedema of the lungs should be treated with strophantin, hypertonic glucose, blood letting, mercury diuretics, posterior pituitary extract, high glucocorticoid dosage and oxygen.[29]

(b) *Heart.* Intracardial injections and massage if necessary.

(c) *Spasms.* Administer phenobarbitone, or Somnifen and muscle relaxants. Von Clarmann[20] suggests succinylcholine; however, according to Durham and Hayes[22] its use is contraindicated. They state that morphine, theophylline, aminophylline and succinylcholine should never be given and tranquillizers only with the utmost caution. Phenothiazines tend to

potentiate the blockage of ChE. With morphine there is a risk of respiratory failure.

Patients should be kept under careful observation for a few days as relapses may occur after initial periods of improvement.

SUMMARY

The more recent knowledge gained as to the symptoms induced by, and the mechanisms and modes of action of different insecticidal organophosphates are discussed. Attention is drawn to the advantages of the use of Toxogonin over those of P₂AM, the most important being that the former reactivator of ChE passes the blood-brain barrier and P₂AM does not, or does so to only a very slight degree.

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