Anticholinesterase Kinetics in Occupational Pesticide Exposure

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ABSTRACT

It is important to evolve bio-monitoring systems in occupational situations as problematic as pesticide exposure. In occupational situations the exposure to pesticide is generally of a subthreshold level, of long standing and to a mixed group of compounds. The organophosphates and carbamates are anticholinesterases, with largely similar biological effects, but with slightly different biotransformation pathways. The validity of the anticholinesterase kinetics is based on specific "selectophore" molecules which take part in the different phases of activation and degradation of the pesticides. The judicial use of critical and precise enzymatic changes, like those of glucronyl transferase with cholinesterase, are supplementary in the biomonitoring of people in pesticide exposure.

In this article the rationale of using the anticholinesterase system is described, and correlations between the broad and general physicochemical structure function relationships of these pesticides and specific biological activity at target sites are illustrated. The secondary reactions such as those of detoxification/inactivation, are an equally important component of any biomonitoring system. Some of these should be incorporated into the study especially of long term/chronic subthreshold exposure, to be able to explain better the late occuring clinical pictures of pesticide exposure.

The basic dilemma of whether "to use" or "not to use" pesticide has long been solved. This has happened, thanks to (i) the pest free/control life-style adopted by nations around the world, (ii) the boom in productivity of large numbers and greater quantities of pesticides, and (iii) the awareness of people at large, with the help of media coverage. The second question now is — how much and for how long will each pesticide which comes into being, be used by whom, and under what safety procedures. The U.S. Environmental Protection Agency, the WHO and ILO joint ventures, and some well renowned European State Agencies have made laudable attempts to effectively implement control procedures for each of the thousands of pesticides synthesised each year. So the third question now being asked is "why" one pesticide and not the other ? Every country has a wide variety of pesticides to choose from and the occupational exposure of the people who manufacture and use these is finally assuming an importance in proportion to the problem.

The basic requirement, function and desirable properties stem from a few biochemical reactions. They belong by and large within the age old classification of pesticides as organochlorine, organophosphorous and carbamic acid esters, and their biotransformations. The purpose of this paper is to elucidate the current approach to bio-monitoring, in an occupationally exposed population, by illustrating the importance of the organophosphatase-cholinesterase system, in mammals and man. People who are chronically exposed to subthreshold levels of the organophosphates are those working as labourers, sprayers and mixers in agricultural and health departments on a regular basis. It also affects those living near orchards and farm lands. This paper predominantly deals with chronic low dose effects, rather than classical acute toxicity. By following the kinetics of the cholinesterase system, it is hoped to justify its regular estimation as an early bio-monitoring programme.

THE HISTORICAL DEVELOPMENT OF THE ANTI-CHOLINESTERASES

The organophosphates gained recognition as compounds of war during World War II. The discovery by trial and error of their function of paralysis led them to acquire the popular name of nerve gas and a new category of war by chemical warfare emerged[2]. The synthesis of these compounds took place much earlier in the 1888's by Lassaighe and Moschinin[3] who synthesised simple forms and tetraethyl pyrophosphate (TEPP) the parent compound. Schrader[1] developed a series of fluorine containing esters and thio-nophosphorous esters including parathion and its oxygen analogue paraoxon, and noted its' toxic effects in the early 1940's. It was from 1950 to 1960 that the era of organophosphates led to a tremendous amount of work on their properties, commercial value and biological uses.

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The non-biological uses of triaryl esters of phosphoric acid, as plasticising agents, as additives to extreme premier lubricants in hydraulic systems, and as lead scavengers in gasoline, have led to occupational exposure with resulting polyneuritis. The phospho-creoste compounds led to severe polyneuritis, when used for treatment of pulmonary tuberculosis in 1899. Epidemic outbreaks of neuropathy were also well recorded in Jamaica, as Ginger Jake Paralysis, by a potent neurotoxin, an isomer : tri otho cresyl phosphate or TOCP. Sporadic episodes are reported now and again, due to contaminated cooking oil or salad oil.

Carbamic Acid Esters have a more natural history based on the extract of the ground beans from the Calaber plant (Physostigma vanerosum). The first potent carbamate insecticide synthesised was Dimetan, and in 1953 the most popular of them, the carbaryl or sevin group was produced. Its popularity is based on its low mammalian toxicity, its environmental degradability and its toxicity to a broad spectrum of insect pests. The toxicological properties depend upon their optimal structure activity having the closest spatial similarity to acetyl choline.

Though most of the insecticides emerged by a process of trial and error, Fukuto[4] was the first to demonstrate the necessity of introducing "selectophore groups". These are specific reactive groups of the molecule, which will allow exploitation of the differences between the fundamental biochemistry of insects and mammals.

KINETICS OF THE ANTI-CHOLINESTERASES

The selectophore groups of the anticholinesterase pesticides interacting with biochemical systems, are dependant on the physicochemical and biological interactions that can take place.

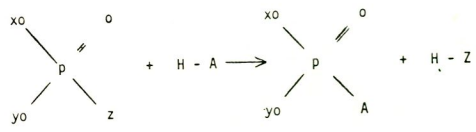
1. Physicochemical Interactions

The biological potency of a pesticide is dependant upon the following physicochemical interactions :

1. Penetration and distribution to various tissues of the body.
2. Biotransformation, where both activation as well as degradation can occur in vivo.
3. Interaction with a structural or functional target site in a tissue.

Most biologically active lipid soluble neutral esters of the pentavalent phosphorous acids are derivatives of either phosphoric I, phosphoric II and phosphoric acid (III), anhydrides or sulphur containing analogues. The influences of various substituent groups can be exerted in the following ways : (a) steric (b) electronic (c) hydrophobic.

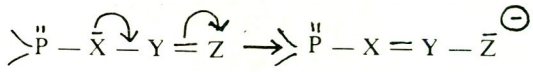
a. The anticholinesterase activity of a compound depends largely on the phosphorylating ability of the esters containing an easily displaced Z substituent. This Z group may consist of fluoride, cyanate, thiocynate, mercaptide phosphoxyloxy aryl or heterocylic groups. The substitution occurs through Schrader's (9) "acyl rule" by which the anhydride linkage between the substituent group can be broken.



The Phosphorylation Complex

The larger the substituent group, the bulkier the aryl oxy group becomes, forming an enzyme-inhibitor complex, the greater as a result, the steric interference with the overall phosphorylation complex. A good correlation was also established by Hansch and Deutsh[7] between the logarithm of the inhibitor constant Ke and Tafts steric constant E.

b. Clark et al (I) describe the atomic reconfigurations as the P-XYZ systems, where XYZ are atoms, different from those used by Schrader. In their scheme the electrons of the P - X bond are accepted by Z, which becomes electron negative thereby remitting in hydrolysis of a weakened P - X bond as follows :



The electron withdrawing or donating properties is described by Hammetts[6] constant[8]. A logarithmic correlation has been observed between the molar concentration of inhibitor necessary to produce 50% inhibition (150) of fly brain acety cholinesterase for a series of meta and para substituted diethyl, ophenyl phosphates.

c. A large number of chemicals with unrelated structures have similar biological properties, in producing narcosis and sleep. A Hydrophobic Model based on the oil/water partition coeffi-

cient was introduced by Meyer and Overten, to explain these similarities. A π constant was defined by Hammett as P_x

$$\pi = \log P_x / P_H$$

The Hydrophobic Model

Where π is a free energy constant, P_H is the octanol water partition coefficient of the chemical and P_x is the value for the modified altered structure. This has been used effectively for estimating the influence of changes on the distribution, penetration, interaction or binding of a phosphorylated molecule at the membrane site, at a synapse or active sites of the enzyme cholinesterase.
In summary the above three equations lead to a single integral equation which considers the total physicochemical response as follows :

$$\text{Biological response (BR)} = f(\text{hydrophosbicity}) + f(\text{electronic}) + f(\text{steric})$$

These fundamentals of organophosphate chemistry have been derived, by the work of many scientists, over many decades and longer. They identify the precision and extremely specific reactions that can occur, within the framework of the "selectophore" concept.

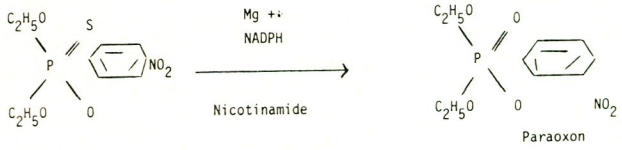
2. Biotransformation with Cholinesterase

The organophosphates and carbanates follow the general metabolic pathways in the following way :

Phase I (Nonsynthetic)		**Phase II** (Synthetic)
Pesticide	——— Metabolite	⟶ Excretory Product
Oxidation		Glucuronidation
Reduction		Sulfation
Hydrolysis		Acetylation
Transfer		

Like most organic pesticides, the anticholinesterases undergo oxidation by the help of mixed function oxidases, their co-enzymes nicotinamide adenine dinucleotide NAD — NADPH system and (2) P — 450 cytochrome system in the case of organophosphate, and P — 420 in the case of carbamates in the Phase I reactions.These two systems of oxidation provide the necessary oxygen and electrons to convert the pesticide into polar compounds. The importance of this step lies in the catalysis and removal of aryl and alkyl groups and the replacement of the sulphur in phosphorothionate esters.

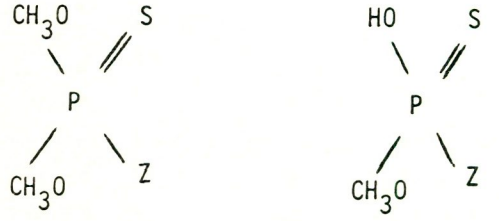
Parathion is a selectively tonic substance, which is by itself a rather weak pesticide but is made into several hundred fold more potent an anticholinesterase agent by the tissue enzymes due to active desulphuration. When parathion is incubated with liver microsomes, in the presence of Mg ions NADPH and nicotinamide, it converts to paraoxon (O, O-diethyl — O — 4 — nitrophenyl phosphate).



While this occurs in all cells of the body, the susceptibility of animals to poisoning by the phosphorothionate insecticides is dependant upon :

i) The rates at which the oxygen analogues are available to inhibit cholinesterase at critical sites in the nervous tissue, particularly synapses (fig. I).

ii) The dynamic relationship between the activation and inactivation of these chemicals by liver tissue enzymes (fig. II). The formation of paraoxon, and increase in potency, is equally matched by a dramatic decrease in anticholinesterase activity and toxicity through the removal of one alkyl group by oxidative dealkylation.



Similar dealkylation of the anticholinesterases also occurs in the post microsomal fractions of the liver. These utilise the enzyme system, glutathione transferase resulting in detoxification by dealkylation, for dichlorvos methyl paraoxon, methyl parathion etc.

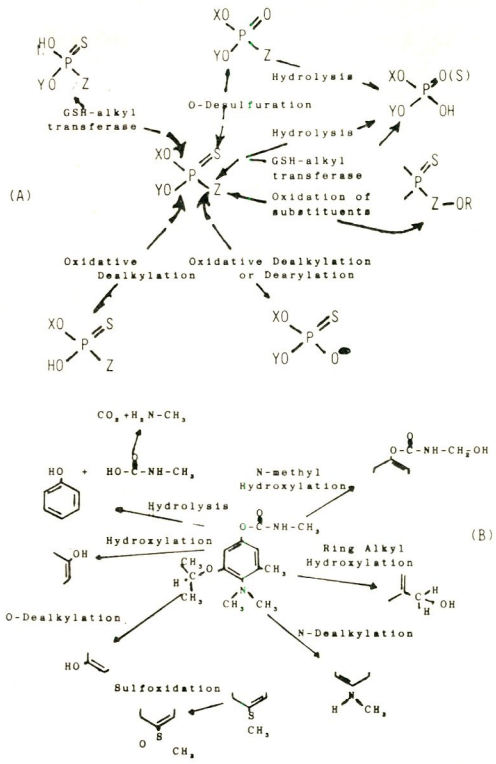


FIG. 1 *A Schematic diagram of various phase 1 Detoxification reactions by (A), the organophosphates, and, (B) by carbamate esters*

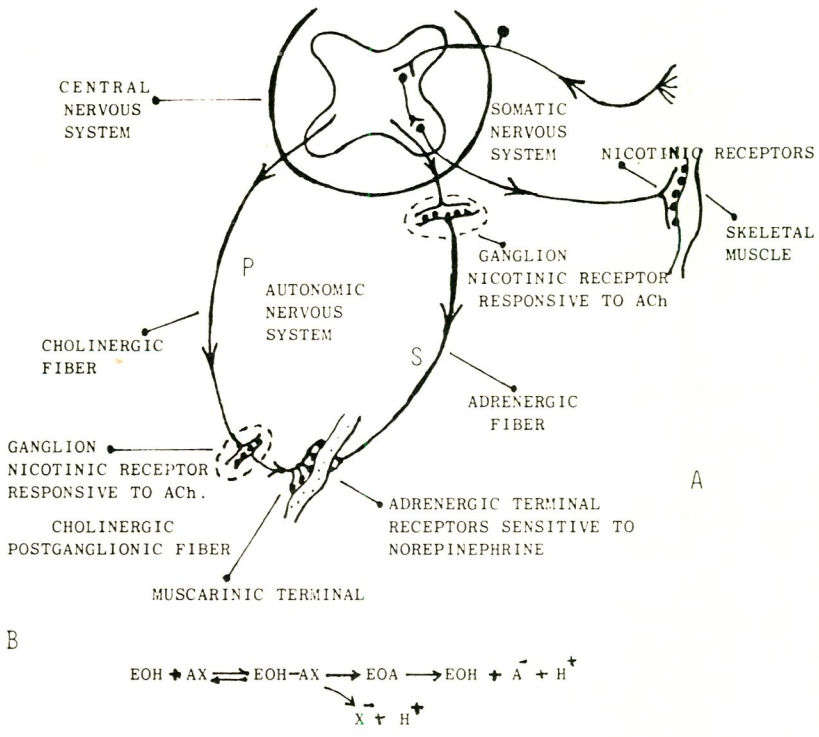


FIG. 2 *A simple diagram showing the various locations of Acetylcholinesterase (AChE) molecules (A), and (B) the mechanism of interaction between A carbamate ester (AX) and AChE.*

A substance may increase the cytochrome P_{450} concentration and the activity of the N-demethylase, whereas another substance inhibits the activity of the N-demethylase although the concentration of cytochrome P_{450} is increased. This principle illustrates the specificity of the microsomal damage enzyme induction, and can occur together with liver damage or without (5).

Similarly the protective properties of phenobarbital, in the delayed phase of regeneration of ChE levels have been suggested, by Purshottam (8).

These depend upon whether the detoxifications have been brought about by a single step detoxification as with dichlorvos, or are based on conversion and subsequent detoxification as with parathion.

CONCLUSION

In illustrating the significance of some of the metabolic functions of the two groups of pesticides, the organophosphates and carbamates, the purpose has been to identify the importance of the following :

1. Cholinesterase is a prime bioindicator in early toxicity. Being a small plasma protein with a half life of one day it responds more promptly to adverse or favourable changes in metabolic functions, especially specific anticholinesterases.
2. Cholinesterase is a selective biomonitor system for subthreshold long term exposure. For each different type of anticholinesterase used the specifity of the enzyme system utilisation, for potentiation and detoxification is important in long term exposure and resynthesis of cholinesterase. It's relationship with "neurotoxic protein" and "neurotoxic esterase" is yet to be established in synaptosomal membranes, from spinal cord and brain tissue.
3. Glucuronosyl transferase is an important indicator of detoxification reactions, whether for short term or long term monitoring in multiple pesticide exposure. The changes of this enzyme system when measured concurrently with cholinesterase, give a better understanding of the kinetics of potentiation and detoxification in exposure to organophosphate and carbamate exposure.

In this paper an attempt has been made to establish the validity and the rationale of the primary and supplementary biomonitoring techniques in the case of anticholinesterases.

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