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Review
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WHAT IS TOXICOLOGY?

A discussion of scientific developments in relation to practical needs

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It is a great honour and pleasure to receive this award. I accept it in recognition of all of those scientists involved in the collaborative research between this Institute and the Medical Research Council Toxicology Unit, UK, spanning 30 years which has stimulated much other research by its dissemination in the international literature. I shall treasure this award as I treasure the friendships developed during this collaboration.

The European Science Foundation through its committee the European Medical Research Council (EMRC) has fostered a programme for the encouragement of European collaboration in research in toxicology and I have been privileged to take part in this activity for the last eight years. Although the Yugoslav Academy of Sciences and Arts is a member of the European Science Foundation it has not joined the European Medical Research Council. Nevertheless the collaboration between this Institute and the Medical Research Council Toxicology Unit predates this initiative by the EMRC and is in the best tradition of European scientific collaboration.

The collaboration began early in the 1950's by a visit to Carshalton by Dr. Vouk who was at the time working in University College, London. This contact made by Dr. Vouk illustrates the need to be able to cross the boundaries of several disciplines. Dr. Vouk, as Director of this Institute, was a physical chemist working in the Department of Physiology in

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A lecture presented oi October 11, 1984 at the Institute for Medical Research and Occupational Health, Zagreb, Yugoslavia on the occasion of the presentation of a plaque and testimonial to celebrate the 35th Anniversary of the Institute.

University College, London and came to talk to us about training of staff and collaborative research in mechanisms of toxicity. Since that time we at Carshalton have enjoyed many visits and long periods of research with Drs. Vandekar, Reiner and Pleština.

Toxicology is the study of toxic substances and toxicity is the change in structure and/or function of a living organism. This definition is of course very broad with respect to the range of possible biological organisms, the nature of the toxin and the biological response. In addition, the expertise required in problems ranging from the practical to the academic is equally wide. Different people who are justified in saying they are concerned with toxicity and toxicology will provide very different answers to the question »What is toxicology?«

Over the last 50 years problems have changed in emphasis. In the past exposure occurred and medical supervision detected undesirable consequences and steps were then taken, if possible, to reduce exposure. Nowadays modern society demands to be assured that a substance may be used safely before exposure to man occurs.

The solution to practical problems involves many scientific disciplines and it is my aim to illustrate the connection between academic and practical toxicology.

The biological activity of chemicals, whether it results in toxicity or not, may be viewed as four phases following absorption of the compound (Fig. 1; ref. 1): (a) Delivery which concerns all those factors which affect the movement of the chemical or its activated metabolite to the site of action. This phase contains many processes including metabolism leading to detoxification or activation, secretion, excretion and distribution into tissues or binding to macromolecules; (b) Primary reaction at the target. Targets are analogous to receptors in pharmacology and physiology. They may be macromolecules, membranes etc. and their properties are modified by reaction with the toxic chemical. (c) Biochemical, physiological and morphological consequences of the primary reaction. (d) Consequence to the organism. Some of the processes in (c) define the nature of the toxicity which is seen as clinical signs, symptoms or syndrome.

This scheme is applicable in general terms to the toxic process for any toxin, for any organism and for any type of toxicity. In practice toxicity is often more restricted and in this lecture I shall be concerned with the toxicity of small molecular weight chemicals to mammals.

It is also important to point out that reaction with some macromolecules does not lead to toxicity. Sometimes even if biochemical consequences of such an interaction can be detected, these events cannot be regarded as toxic.

Dr. Milutin Vandekar visited Carshalton in the 1950's and in addition to working on other topics he worked with Dr. Heath on the toxicity of Metasystox. They discovered that a concentrated solution in water in-

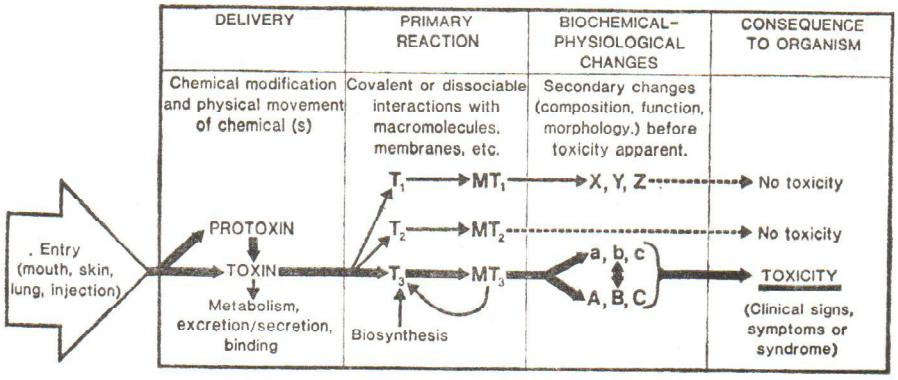

Fig. 1. *Scheme illustrating phases of developing toxicity following entry of chemicals into an organism. Heavy lines and letters lead to toxicity whereas light lines are events which are unproductive. T_1, T_2, T_3 and MT_1, MT_2, MT_3, are targets before and after modification by the chemical respectively and A, B, C, X, Y, Z, a, b, c, represent an undefined and often unconnected number of changes which may or may not be sequential. Acute and chronic toxicity may be differentiated by the time in one or several of the phases. Recovery from changes in function or morphology can occur by regeneration of T_3 and by disposal and repair of modified tissue. Prophylaxis or therapy is theoretically possible in several of the phases (reproduced from Trends in Pharmacological Sciences cf. ref. 1).*

creased in toxicity rather rapidly. Some of their findings are illustrated in Fig. 2. They showed that two molecules interacted so that a methyl group was transferred from one molecule to the sulphur atom in the other to produce a sulphonium compound with a positive charge on the sulphur atom. Both the toxicity to rats and the *in vitro* inhibitory power of Metasystox are increased by this reaction approximately 1000 times. In two substantial papers the generality of this proposition that sulphonium compounds are more active than the parent compounds was demonstrated (2). This finding illustrates that resemblance of organophosphorus compounds to acetylcholine leads to high anticholinesterase activity; the two most important features are that the distance between the positive charge and the P=O group should be similar to that distance between the positive nitrogen and the C=O in acetylcholine.

Dr. Elsa Reiner has visited Carshalton many times and during her last visit in 1980/81 initiated new studies which are now continuing and developing into a wider research programme (3). I illustrate one early study on the pH dependence of the spontaneous reactivation of carbamylated and phosphorylated acetylcholinesterase (Fig. 3; ref. 4). This pH dependence shows a close resemblance to that for the hydrolysis of acetylcholine by the same enzyme. Other studies (5) finally established the for-

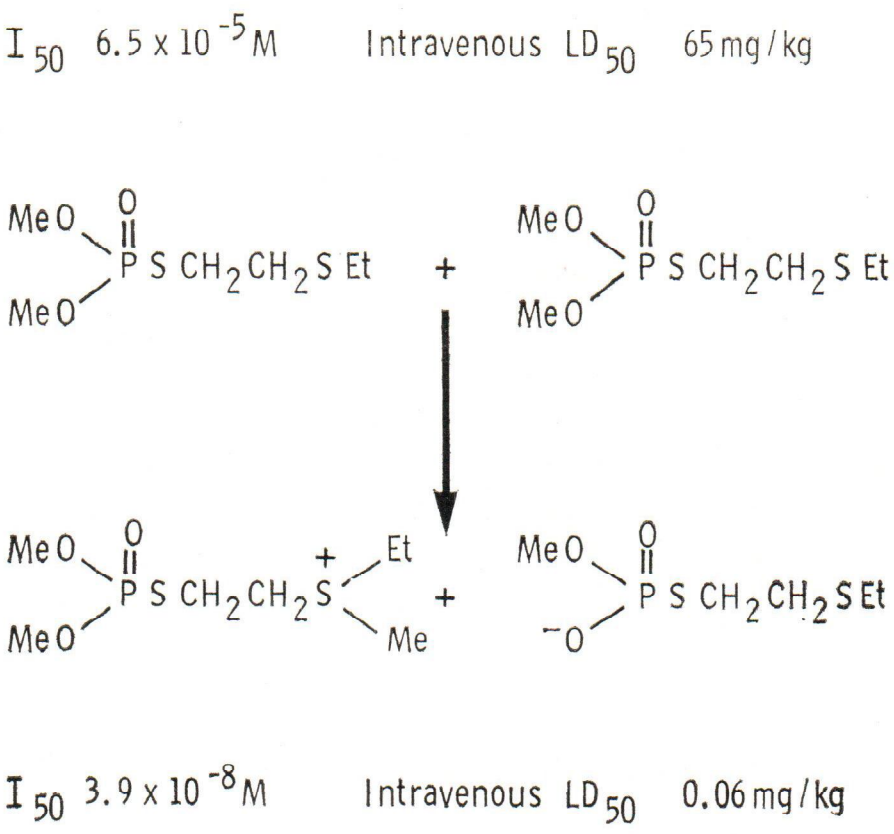

Fig. 2. *The non-enzymic conversion of Metasystox to a sulphonium compound (cf. ref. 2).*

mal analogy between the reactions of organophosphorus compounds and esters of carbamic acid with esterases and the esterase-substrate reaction (6). At an international meeting initiated and organised by Dr. Reiner in Split in 1975 the three possible reactions of phosphorylated esterases were defined (Fig. 4; ref. 7). One reaction is a slow return of the original catalytically active enzyme. This reaction may be accelerated by the nucleophilic oximes which were developed rationally from knowledge of the reaction of enzyme and inhibitor and have proved to be effective therapeutic agents. In what is known as the aging process the reaction can take place by two mechanisms, one involving the break in the P-O-R group between the R and O and the other between P and O. The product of these two different reactions is the identical phosphorylated esterase

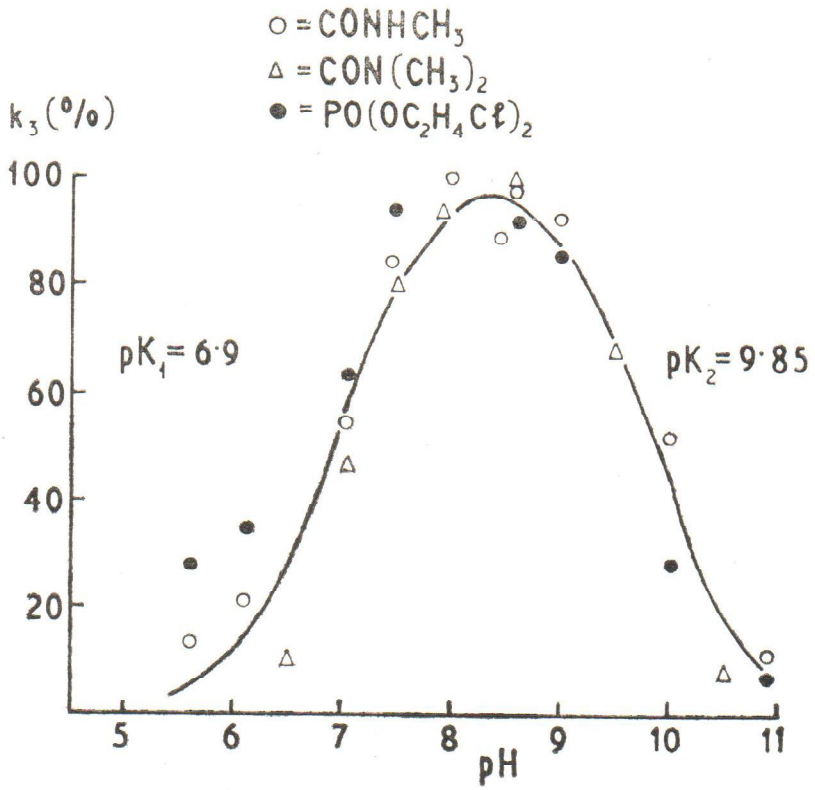

Fig. 3. *Influence of pH on spontaneous reactivation of carbamylated and phosphorylated acetylcholinesterase (cf. ref. 4).*

containing a P—O⁻ group. These reactions have been shown to occur with a variety of organophosphorus inhibitors and serine or B-esterases.

Dr. Radovan Pleština has been involved in the trials of the use of Metrifonate for the treatment of infections of *Schistosoma haematobium*. Metrifonate is an organophosphorus compound which by a chemical and non-enzymic reaction changes into Dichlorvos, a direct and highly active inhibitor of acetylcholinesterase (Fig. 5; ref. 8, 9). Two or three doses of 7—12 mg metrifonate is sufficient to reduce the population of worms as shown by excretion of eggs in the urine. During the preliminary trials of this method of treatment the activity of the cholinesterases in blood was determined (Fig. 6; ref. 10). It is instructive to consider, with our present knowledge of the reactions of esterases with organophosphorus compounds, what we can derive from these results. Plasma cholineste-

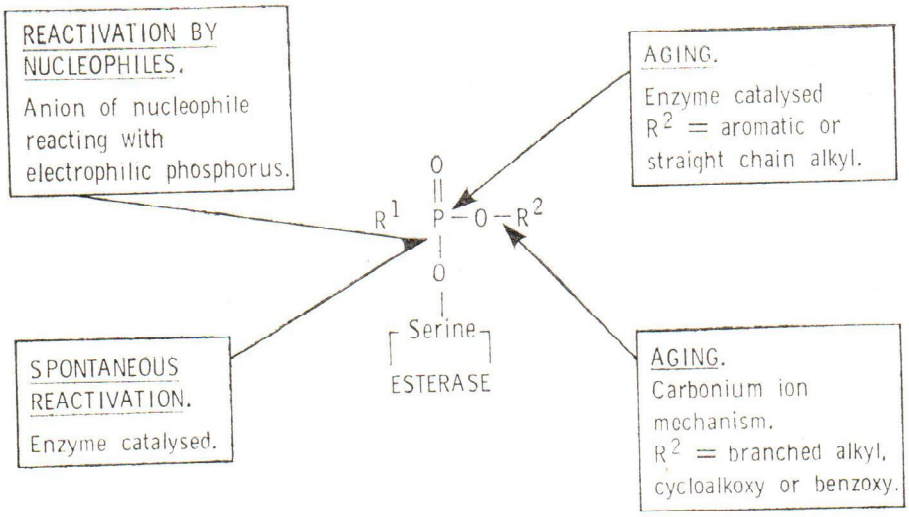

Fig. 4. *Properties of phosphylated esterases (cf. ref. 7).*

rase is more inhibited than erythrocyte cholinesterase. Plasma cholinesterase activity returns faster than erythrocyte cholinesterase. This is because although the inhibited cholinesterase is stable, new enzyme is resynthesised, probably in the liver. Metrifonate and dichlorvos are dimethoxy compounds and a dimethylphosphorylated acetylcholinesterase is produced which is relatively unstable with a half-life of approx 51 min. (11).

The inhibited enzyme also ages and loses one of the methyl groups and this transformation has a half-life of 3.8 h. When metrifonate is slowly transformed to dichlorvos the conditions approximate to a continuous infusion and the proportion of aged enzyme increases. Thus under these circumstances measurements of erythrocyte cholinesterase activity indicate the amount of aged enzyme present. From Fig. 6 the rate of return of erythrocyte cholinesterase activity is rather slow ($^1/_2$ life about 60 days); this is the rate of replacement of old erythrocytes by resynthesis of new ones. It is usually accepted that after exposure to organophosphorus compounds measurements of erythrocyte cholinesterase activity is a monitor of the expected activity at other sites such as myoneural junctions and the central nervous system. This is certainly true for acute exposure but there are instances, due to higher rates of resynthesis of new enzyme, when inhibition of erythrocyte cholinesterase may be an overestimate of inhibition at other sites.

The cholinesterases in plasma and in erythrocytes or brain are different enzymes and inhibition of the former appears to have no toxicological consequences. Nevertheless inhibition of plasma cholinesterase after exposure to organophosphorus compounds indicates that some has been absorbed. Indeed with a knowledge of the second order rate constant for the reaction *in vitro* of plasma cholinesterase with an organophosphorus compound, the exposure of the enzyme *in vivo* may be calculated. This principle has been applied by *Ehrenberg and colleagues* using the long lived protein haemoglobin in erythrocytes (12—14). Many electrophilic reagents are toxic and react with nucleophilic centres in a variety of macromolecules. In haemoglobin such centres are cysteine, histidine and lysine. With modern developments in analytical chemistry it is possible to measure extremely low concentrations of derivatives of these amino acids (14). The long life of the haemoglobin means that after an acute exposure the received dose can be evaluated several weeks later. It must be emphasised that the low proportion of modified resi-

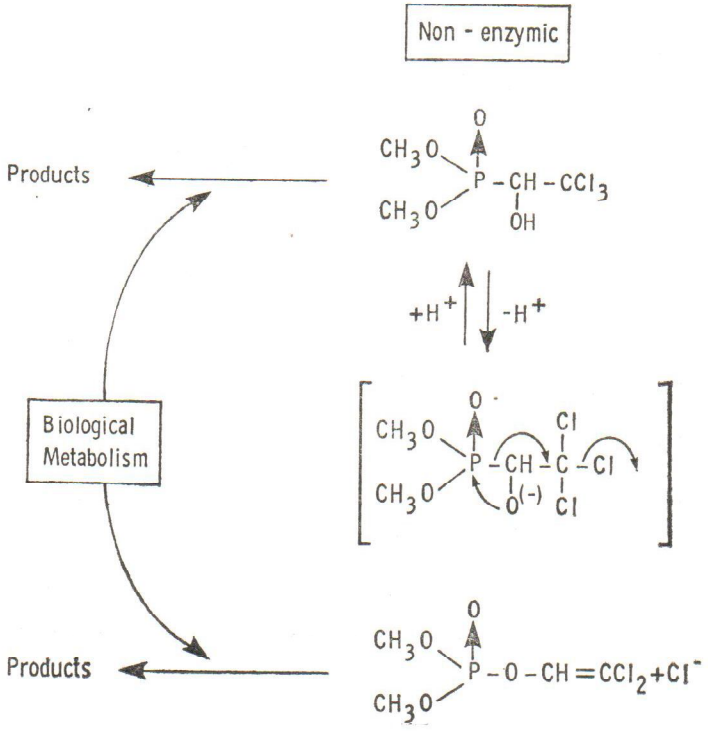

Fig. 5. *The non-enzymic conversion of Metrifonate into Dichlorvos (cf. ref. 8).*

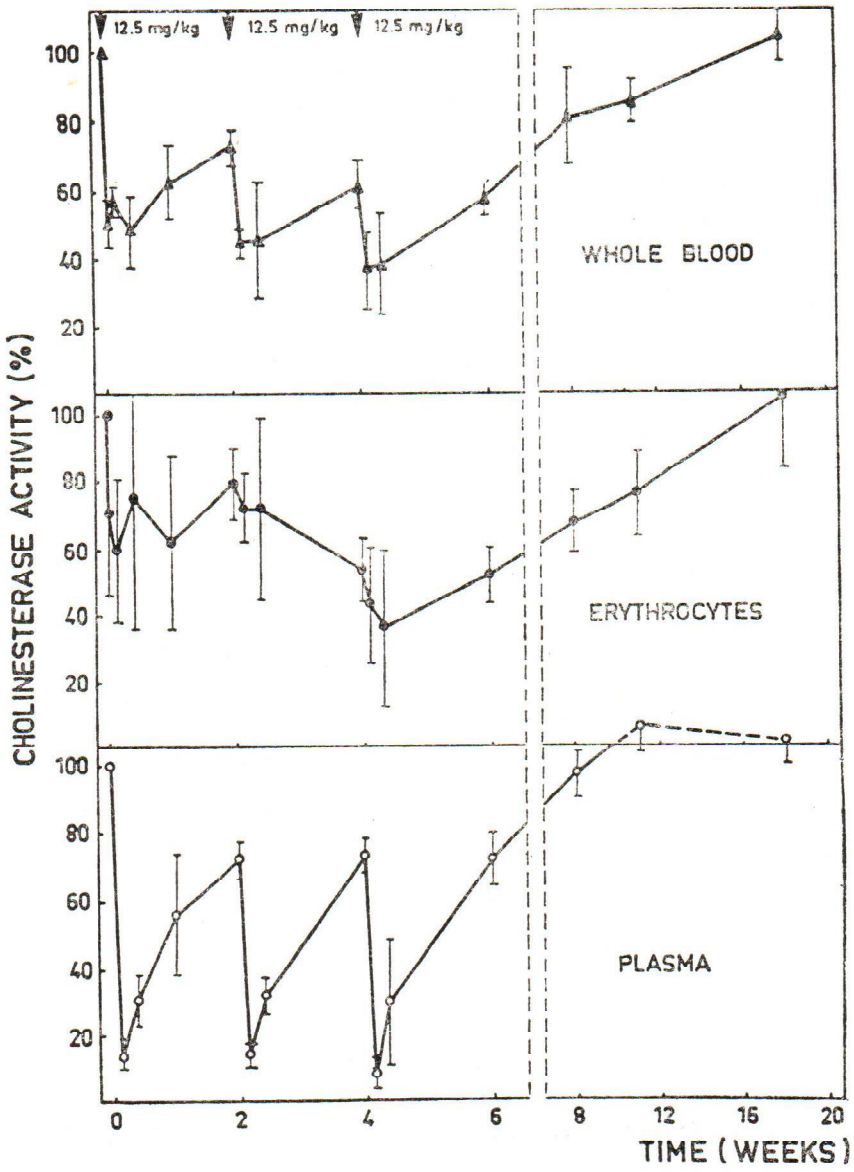

Fig. 6. *Cholinesterases in blood of children treated for schistosomiasis with Metrifonate (cf. ref. 20).*

dues in haemoglobin is of no toxicological significance except as a measure of exposure. These developments are of obvious importance for prospective epidemiology and in addition will allow dose-response relationships to be established for exposure of humans. The general principles of this procedure are shown in Fig. 7. There are of course problems of interpretation for compounds which are activated *in vivo* to the proximal toxin.

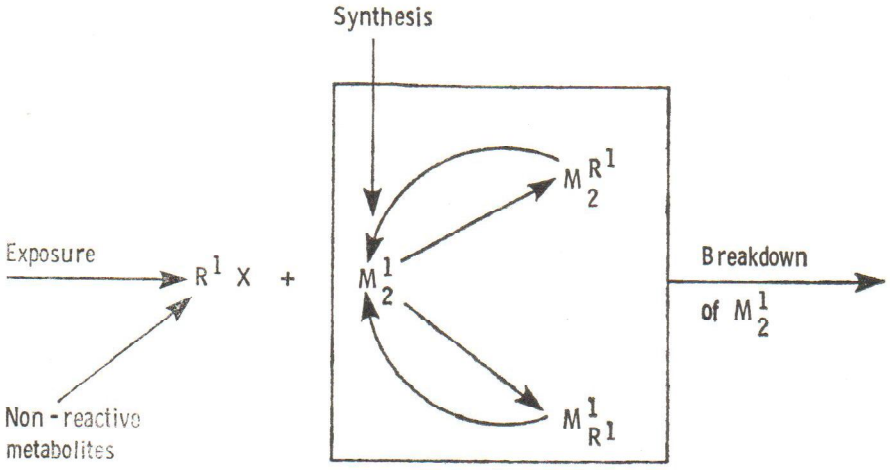

Fig. 7. *General principles of the use of haemoglobin as a monitor of received doses of electrophilic chemicals (cf. refs. 12—14).* M^1_2 *is the macromolecule haemoglobin with two nucleophilic centres.*

It has been known for a long time that certain organophosphorus compounds cause a delayed neuropathy in man. This undesirable property must of course not be allowed to occur in commercial pesticides. The best experimental animal is the chicken and the disease is shown in man by unsteadiness of gait often followed by complete paralysis particularly of the legs and less severely in the arms. From histological examination the condition is known to be caused by an axonopathy brought about by a dying back lesion in the long axons in the peripheral and central nervous system (particularly the spinal cord). Often the clinical condition is permanent. The primary target which initiates the disease process is an esterase present in the nervous system known as neurotoxic esterase or more recently neuropathy target esterase (NTE) (15—17). It has been established that for the disease to occur NTE must be phosphorylated followed by the aging process as defined above (Fig. 8). If, as with some inhibited NTE's (e. g. phosphinylated), aging cannot occur, the disease does not develop. The aging process for NTE is unusual and maybe u-

nique for the released group which normally is found in the aqueous medium is transferred to another site adjacent to the catalytic centre (18). The physiological function of NTE is unknown even though it is now known to be present in other tissues (e. g. lymphocytes). This research has allowed the structure-activity relationships at the target to be determined and for quantitative prediction of hazard for new organophosphorus compounds. It has also been possible to approach rationally the problem of chronic exposure. In chickens it seems likely that although 80% or more inhibition of NTE after a single acute dose will lead to delayed neuropathy, chronic administration with 50% inhibition of NTE can be tolerated for 10 weeks and may be for ever (19). As shown in Fig. 9 this is not because the chicken has become tolerant to the com-

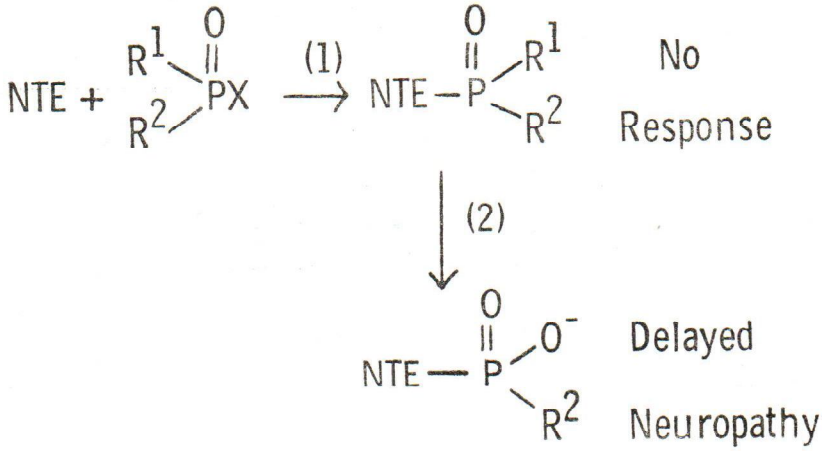

Fig. 8. *Primary chemical interaction leading to delayed neuropathy. NTE = neuropathy target esterase. When R_1 and R_2 are directly linked to the phosphorus atom by P-C bonds as in phosphinates only reaction (1) can take place and delayed neuropathy does not develop. For the same reason reaction of NTE with carbamates and sulphonates does not lead to neuropathy (cf. refs. 15—17).*

pound because a larger dose causes the disease. Thus knowledge of the target (NTE) in this disease caused by certain organophosphorus compounds has allowed a real quantitative appreciation of potential hazard to be attained; this is a great advance on the limited conclusions which can be reached by dosing and observing chickens.

In my previous discussions I have concentrated on examples when the primary target involved in the development of toxicity has been identified (Fig. 1). For acute toxicity of organophosphorus compounds

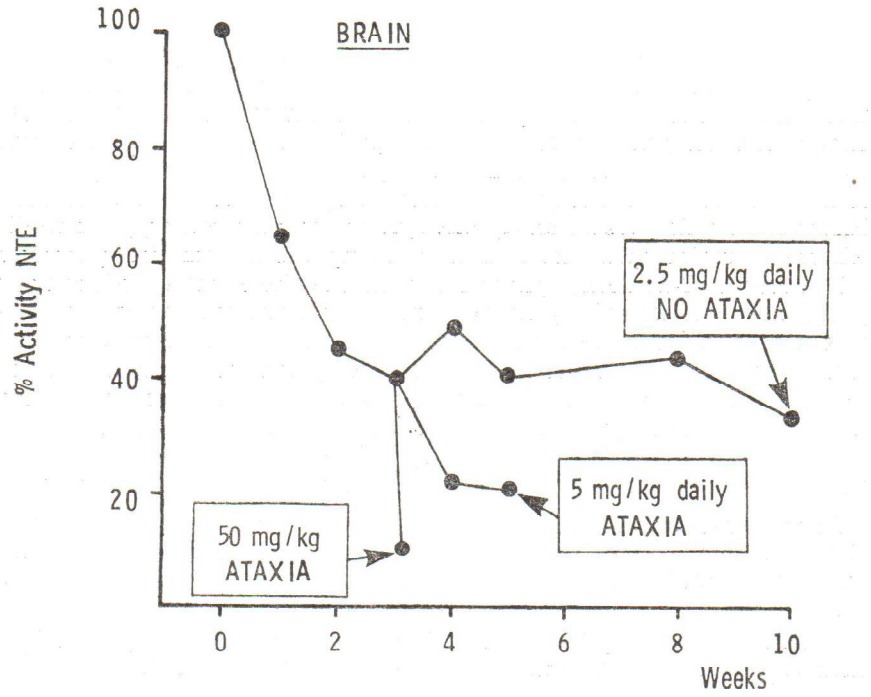

Fig. 9. *Chronic dosing of an organophosphorus compound, inhibition of neuropathy target esterase (NTE) and the development of neuropathy. The compound used was mono 2-methylphenyl diphenyl phosphate (cf. ref. 19).*

the target is acetylcholinesterase and for the chronic delayed neuropathy, neuropathy target esterase (NTE) is the target (Fig. 1; T_3). I have also pointed out that there are many »targets« which react with chemicals and do not cause toxicity or disease (Fig. 1; T_1 and T_2).

Dr. Pleština when he visited Carshalton worked on the effects of pyrroles on the lung. These pyrroles are the reactive products produced by *in vivo* bioactivation of pyrrolizidine alkaloids (20). It was concluded that the primary target in the lung was the endothelial cells on the blood side.

In Carshalton we have been studying the toxicology of certain impurities in organothionophosphate pesticides which became of interest after the poisoning episode in Pakistan in 1976. Although there is little doubt that the main cause of this episode was the presence of isomalathion in the 50% water dispersible powder malathion preparation, other substances were present (21). Of these OSS-trimethyl phosphorodithioate was more effective than isomalathion in inhibiting the carboxyleste-

rase responsible for the detoxification of malathion. However OSS-trimethyl phosphorodithioate is also rather toxic and produced late deaths unexpected for a water soluble organophosphorus compound (21). The cause of death is due to lung damage (22, 23) and the resultant respiratory insufficiency (24). Morphological electronmicroscopic studies have shown that the primary target is the type 1 pneumocytes (25). As knowledge of the properties of some cell types in the lung has improved, it is now possible to identify the primary target by biochemical methods. Endothelial cells possess an uptake system for 5-hydroxytryptamine (26) whereas alveolar epithelial cells have an uptake system for putrescine (27). After OSS-trimethyl phosphorodithioate there is a reduction of putrescine uptake by lung slices from rats a few hours after dosing. At this time and for more than 2 days the uptake of 5-hydroxytryptamine is not decreased (24). Thus we see the development of biochemical knowledge of differentiated cells in the lung is allowing us to reach decisions about the cellular specificity of toxins. In this example I have given an example of a target (Fig. 1; T_3) being defined in terms of the cell type: the macromolecular target in these cells has not yet been identified.

In my final example I wish to discuss the neurotoxicity of certain chemicals and to show that a major advance has been the identification of the proximal toxin i.e. the delivery of the toxin to its site of action (Fig. 1).

Hexane, methylbutylketone and 2-hexanol cause a mainly peripheral neuropathy in man and animals. It has now been shown that a common

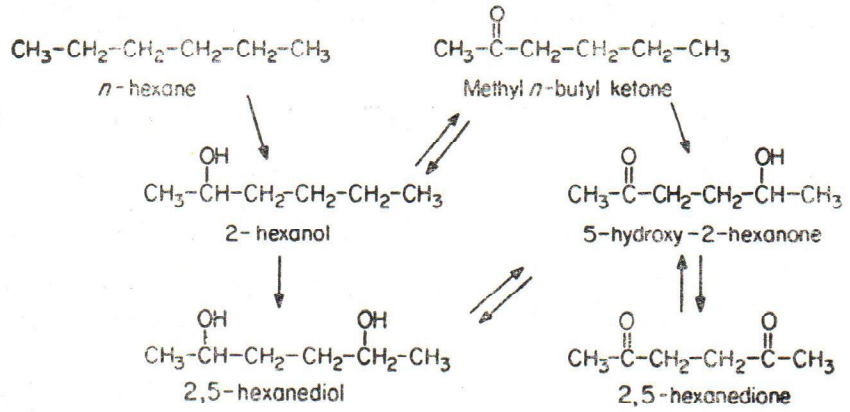

Fig. 10. *Metabolic pathways leading to the formation of 2:5-hexanedione (cf. ref. 28).*

metabolite is 2,5-hexanedione and this is the proximal toxin (Fig. 10; ref. 28). A collection of results from several authors has shown that the structure essential to cause neuropathy is a compound with the two keto oxygen atoms separated by four carbons (Fig. 11; refs. 28—33). Other compounds which may be metabolised to compounds with such a structure also cause neuropathy. Thus by a study of the processes of meta-

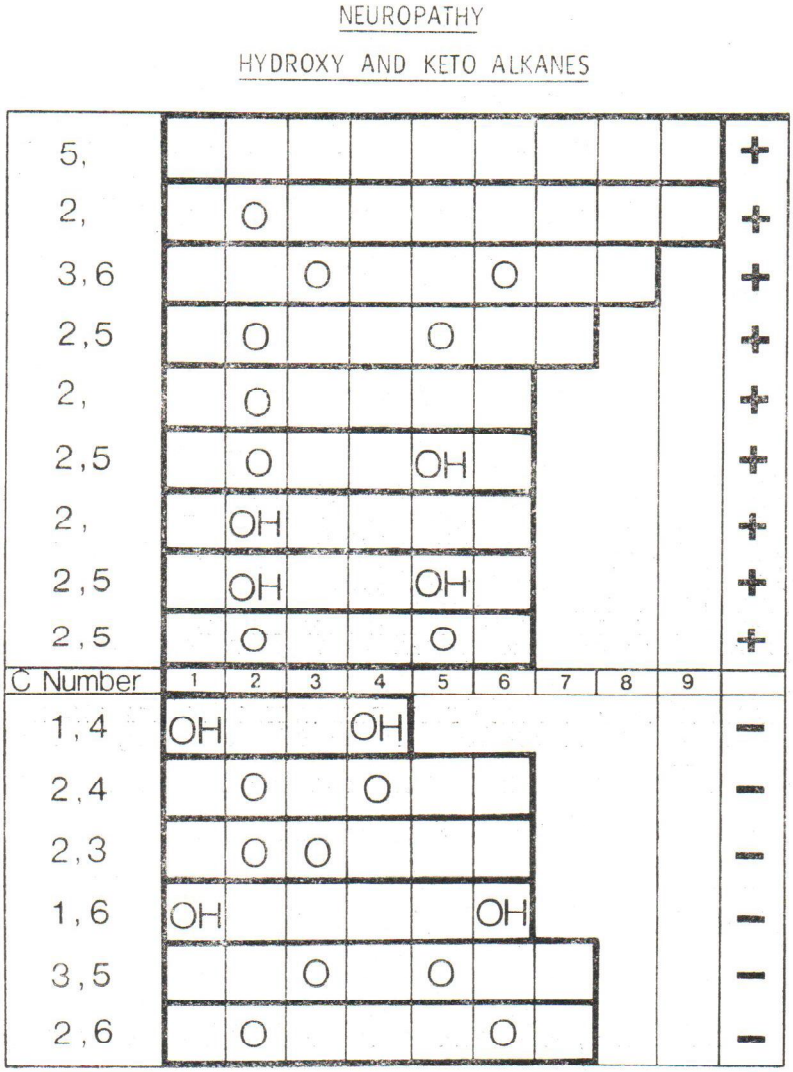

Fig. 11. *Structure activity relationships in the production of peripheral neuropathy by hydroxy and keto alkanes (cf. ref. 28—33).*

bolism one hypothesis has linked together several different chemicals causing the same toxicity.

A recent paper (34) has provided evidence that the chemical basis for their action may be reaction with the e-amino group in lysine to form a pyrrole derivative. A dimethyl derivative (3,4-dimethyl-2,5-hexane dione is more neurotoxic than 2,5-hexanedione and is also more reactive with e-amino groups of lysine to produce a pyrrole (35, 36). These results suggest the nature of the target (Fig. 1; T_3) although many irrelevant targets undoubtedly exist (Fig. 1; T_1 and T_2).

In trying to answer the question »What is toxicology« I have tried to present toxicology as a meeting place of many scientific disciplines. The drive for more understanding of the relationships between chemical, biochemical, physiological and morphological events leading to the final clinical syndrome will show us that there are many things to measure — these will increasingly be of a biochemical nature. There is no doubt that such advances will allow us to make rational decisions how to use chemicals safely for the benefit of man.

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Sažetak

ŠTO JE TO TOKSIKOLOGIJA

Rasprava o razvoju znanosti u odnosu na praktične potrebe

Povodom dodjeljivanja nagrade Instituta za medicinska istraživanja i medicinu rada, Zagreb, za dugogodišnju suradnju, prikazan je primjerima odnos bazičnih istraživanja i aplikativnih rezultata i istaknuta je dugogodišnja znanstvena suradnja dvaju srodnih instituta. Napravljena je evaluacija nekih istraživanja iz područja toksikologije i prikazana povezanost bazičnih i aplikativnih istraživanja.

Istaknuto je da je ovo područje multidisciplinarno i da je potrebna tijesna suradnja stručnjaka iz mnogih područja znanosti kako bi se utvrdile i razumjele zakonitosti i mehanizmi toksičnog djelovanja otrova.

Toksikološka jedinica Britanskog
savjeta za medicinska istraživanja,
Carshalton, Velika Britanija

Primljeno 5. II 1985.