INSECTICIDE METABOLISM AND ITS INHIBITION IN RELATION TO RESISTANCE

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Summary

Resistance to insecticides in insects is often due to the action of detoxicating enzymes which metabolize the molecule in a variety of ways to form non-toxic products. An understanding of the types of detoxicating reactions and the extent of their availability to different animals can often be used to advantage in killing unwanted species. Where these enzymes can be characterized, they can be selectively inhibited by synergists which can then potentiate the effect of the insecticide in whole animals. The practical and theoretical applications of synergists are manifold. These together with their advantages and disadvantages are discussed.

INTRODUCTION

In the metabolism of a toxic molecule such as an insecticide, the end products can be either non-toxic, less toxic, or may end up being even more toxic than the parent compound. The ability of an insect to survive a toxic environment depends to a large extent upon how efficiently it is able to metabolize and dispose of the toxic components. Insects are able to do this through a wide variety of detoxication mechanisms, operated through a battery of enzymes which detoxify the toxic component in different ways. These reactions, at least in some cases, appear to be special biochemical defence mechanisms developed in the course of evolution, and not concerned with the normal biochemical operations of the animal.

METABOLIC FATE OF FOREIGN COMPOUNDS

According to Williams (1959), a toxic component is often disposed of in two ways. The first step involves a change in the biological action of the compound which is brought about by enzymes that either oxidize, reduce or hydrolyse the molecule, followed by a second step which involves the conjugation of the altered molecule with either a sugar, an amino acid, or a peptide. This step helps to increase the aqueous solubility of the metabolite, thus facilitating its efficient elimination through the excretory system.

Generally most mechanisms are available to insects although some are more active than others. As a result, the same molecule may be metabolized to different components in different species. One example is DDT (see Fig. 1). This insecticide is dehydrochlorinated to DDE to a greater extent than it is oxidized by microsomal enzymes in DDT-resistant flies (Lipke and Kearns, 1960). On the other hand, the same DDT molecule is oxidized very extensively to dicofol in the fruit fly (*Drosophila melanogaster*) (Tsukamoto, 1961). Rats metabolize DDT mainly to DDA

FIG. 1: Major DDT metabolites in various animal species.

and excrete it as a conjugate of either cholanic acid or amino acids (Peterson and Robinson, 1964) with minor amounts of DDE. Microorganisms, on the other hand, reductively dechlorinate DDT to DDD (Baker and Morrison, 1965; Kallman and Andrews, 1963). These variations only reflect the differences in the efficiency of a number of possible routes of metabolism found in different species.

On the contrary, one must bear in mind that not all metabolic reactions result in the detoxication of the toxic molecule, for there are cases where the metabolism of a toxic compound can result in the formation of more toxic metabolites. For instance, in the organophosphorus insecticides where the P → S analogues are metabolized to the P → O analogues by microsomal enzymes, their toxicity is enhanced by the mere fact that they act as better anticholinesterase agents than their parent compounds.

When one or more of these metabolic routes give rise to toxic metabolites in one animal and to non-toxic products in another, considerable variations in the biological activity of the molecule occur, which are often used to advantage by toxicologists to kill selectively unwanted species. In this case, selectivity is due to differences in the metabolic routes in different species. For instance, houseflies are very susceptible to dimethoate (Fig. 2). This is because the phosphatase enzyme which hydrolyses the compound is unable to keep up with the oxidation enzymes which cause the formation of the oxygen analogue which is the toxic metabolite. On the contrary, the same insecticide is non-toxic to vertebrates which

FIG. 2: Some metabolites of dimethoate (broken arrows indicate points of attack by enzymes).

have an extensive amidase activity in their liver (sheep) which detoxifies it to the non-toxic dimethoate acid. This kind of information, relating to differences in the metabolic routes employed in different species that decide the fate of the insecticide, together with an understanding of the mechanisms of detoxication and the extent to which each is available to animals, is extremely useful to the toxicologist, not only in designing new insecticides to kill insect pests selectively, but also in developing inhibitors to inhibit specific detoxication mechanisms to reinstate the effectiveness of the insecticide.

DETOXICATION MECHANISMS IN GRASS GRUBS

In this connection an attempt was made in our laboratory to determine the types of detoxication reactions available to the grass grub (*Costelytra zealandica*) as this insect is one of the major agricultural pests in New Zealand.

These animals have very efficient conjugating mechanisms, one of which is a premercapturic acid synthesizing system (Clark *et al.*, 1967). A premercapturic acid is a glutathione (GSH) conjugate of a foreign compound like dichloronitrobenzene and the reaction can be brought about by an enzyme called GSH-S-aryltransferase. Other compounds that can serve as alternative substrates for this enzyme are organochlorine compounds as well as some alkylating agents like dinitrochlorobenzene (DNCB), nitropyridine-N-oxide and nitroquinoline-N-oxide. Amongst the animals that have been so far surveyed for this enzyme, the grass grub seems to have the highest concentrations of it. However, it is not known if this enzyme is responsible for resistance to organochlorine insecticides, although it can be shown that DDT resistance in grass grubs is certainly not due to it. Other GSH-transferase enzymes have also been found in grass grubs, namely, GSH-S-alkyltransferase and a methyltransferase responsible for the transfer of a methyl group from methyl parathion on to GSH. However, both these enzymes are present in only

trace amounts compared with the amount of aryltransferase available in these animals.

Although grass grubs are not known to be tolerant to lindane they have an efficient enzyme system to detoxify lindane and PCCH (pentachlorocyclohexene) isomers to metabolites identical with S-2,4-dichlorophenyl-GSH (Clark *et al.*, 1969). These enzymes, together with aryltransferase, can be inhibited by inhibitors such as bromophenol blue (BPB) and tetrabromophenolphthalein ethyl ester (TBPEE). At the same time, each of these enzymes can also be distinguished from DDT-ase and selectively inhibited using specific inhibitors (Fig. 3). For instance, lindane metabolism is inhibited by WARF antiresistant but unaffected by tertamethyldiaminodiphenylmethane (TDM), whilst PCCH metabolism in blowflies (*Lucilia sericata*) is affected by TDM and not by WARF antiresistant. On the other hand, DDT-ase activity in flies is affected by both of them, while aryltransferase activity is unaffected by either of them.

FIG. 3: Selective inhibition of glutathione-dependent enzymes metabolizing above substrates.

Apart from using GSH, grass grubs can also use amino acids such as cysteine to conjugate with aromatic acids like benzoic acid, glucose, sulphates and phosphates for conjugating with phenols to dispose of them easily as water-soluble metabolites (Binning *et al.*, 1967).

Another group of enzymes known to occur in grass grubs are the microsomal oxidizing enzymes. These enzymes are highly non-specific in their substrate requirement and therefore can be expected to oxidize almost any kind of foreign compounds. However, in grass grubs the rate of microsomal activity is extremely low. For instance, the rate of oxidation of aromatic methyl groups on compounds such as nitrotoluene is almost twenty times lower than in flies in terms of the weight of a whole animal (Hook and Smith, 1967). Similarly, the rate of oxidation of the alkoxy group in ¹⁴C-labelled "Baygon" was only 12% while the fly was able to oxidize it to 60% as acetone. Thus, if the toxicity of this insecticide depended inversely on the rate of oxidation of this group, one can expect "Baygon" to be more toxic to grass grubs than to flies, provided other factors causing resistance are not involved.

One other metabolic reaction detected in grass grubs by other workers is its ability to detoxify DDT to DDE (Perrott *et al.*, 1966). This reaction has been attributed by them to be responsible for DDT resistance in these animals. On the other hand, this dehydrochlorination reaction has not been so far demonstrated in *in vitro* systems to be due to the enzyme

DDT-dehydrochlorinase (DDT-ase) although occurrence of this enzyme in DDT-resistant flies can be demonstrated and correlated with DDT-resistance in *in vitro* experiments (Lipke and Kearns, 1960). Such experiments are useful in testing and designing synergists to potentiate the effect of the insecticide in resistant species. When an attempt was made to correlate the levels of this enzyme in susceptible and resistant strains of grass grub using ^{14}C -labelled and unlabelled DDT, the work did not prove conclusively the occurrence of this enzyme in either strain.

SYNERGISTS AND THEIR MODE OF ACTION

However, the sorts of metabolic reactions which worry a toxicologist most are the detoxication reactions, particularly when they manifest in the form of a resistance phenomenon in insect pests. It is then he is forced to take one of the many alternatives, either to abandon the use of the insecticide in cases where resistance to the insecticide is overwhelmingly chronic and look for new ones, or use more of the insecticide, provided residue problems can be kept to a minimum. On the other hand, if the insecticide is cheap with slight residue problems, it is profitable to use it in combination with an effective synergist, to overcome both the residue problem and the resistance phenomenon.

Generally an insecticide synergist can be regarded as an enzyme inhibitor that potentiates the effect of an insecticide by interfering with its breakdown by the detoxicating enzyme, but itself having no toxic effect upon the animal. However, although synergists are enzyme inhibitors, not all

FIG. 4: DDT-dehydrochlorinase inhibitors and synergists.

enzyme inhibitors are good synergists. This is because some inhibitors are water-soluble, and therefore cannot penetrate the lipid layers on the cuticle of the insect. On the other hand, synergists are usually lipid-soluble compounds and are therefore able to penetrate the cuticle to arrive at the site of action (Fig. 4). Thus a synergist will only potentiate the effect of an insecticide provided the insect has acquired a resistance to it by developing a degrading enzyme. In such cases the effect of a synergist when in combination with an insecticide will be more than additive. On the other hand, no synergist will potentiate the effect of an insecticide if the mechanism of resistance is due to physiological factors such as development of a thicker cuticle, decreased cellular or neural permeability, decreased sensitivity of the site of action or increased storage in less sensitive tissues, etc.

Sometimes a synergist developed to potentiate the effect of an insecticide may antagonize another structurally unrelated insecticide. Thus WARF-antiresistant, a DDT analogue which was developed as a synergist for DDT resistance, also competitively inhibits microsomal enzymes that are involved in parathion activation (Nakatsugawa and Dahm, 1965). Such compounds cannot be used to synergize insecticide formulations containing a mixture of the two as it would then inhibit the activation of parathion to paraoxon — the potent anticholinesterase agent. At the same time there are synergists that can synergize two unrelated insecticides. For example, TDM which was developed as a GSH analogue to synergize DDT resistance in DDT-resistant flies, was also found to synergize *m*-tertiary-butylphenyl-N-methyl carbamate in flies resistant to the above insecticide (unpubl. data). In theory, such synergists can be used to synergize insecticide formulations containing the two insecticides.

Some synergists can improve the spectrum of activity of certain insecticides. For instance, carbaryl is toxic to bees, but not to flies and body lice. However, a mixture of carbaryl with piperonyl butoxide is extremely toxic to all of them.

Sometimes selection of a resistant strain of insects for a series of generations with an insecticide plus a synergist can result in the production of super-strains containing much higher levels of a synergist-insensitive enzyme. This was found by Oppenoorth (1965) with DDT/chlorfenethol combinations. He showed that DDT-ase enzymes from such insects were less readily inhibited by chlorfenethol than enzymes from flies resistant to DDT alone. In this case, since both the enzymes have an absolute requirement for GSH to carry out their metabolic reaction, synergists analogous in structure to GSH should inhibit both types of enzymes. Indeed this has been the basis for the design of TDM which is an analogue of GSH and its competitive inhibitors (Balabaskaran and Smith, 1970).

Thus, when TDM was tested as a synergist against DDT-resistant grass grubs and flies, it synergized the latter but not the former. This could be expected if DDT resistance in grass grubs did not involve the enzyme DDT-ase. Further, this observation also agrees with the lack of synergism with chlorfenethol in DDT-resistant grass grubs as demonstrated by Perrott *et al.* (1966).

However, the above phenomenon as observed with DDT synergist combinations, must be regarded as unique, since it is not known to occur with other insecticide-synergist combinations. Hence, one should not be discouraged in using synergists in combination with insecticides when it is necessary.

Apart from the practical applications of synergists, the fundamental investigation of their action has not only provided a better appreciation of the mechanisms of detoxication, it has also revealed some of the basic biochemical processes involved in insecticide resistance, and also of the mode of action of insecticides.

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REFERENCES

- Balabaskaran, S.; Smith, J. N., 1970: *Biochem. J.*, 117: 989-97.
 Baker, P. S.; Morrison, F. O., 1965: *Can. J. Zool.*, 42: 324-5.
 Binning, A.; Darby, F. J.; Heenan, M. P.; Smith, J. N., 1967: *Biochem. J.*, 103: 42.
 Clark, A. G.; Darby, F. J.; Smith, J. N., 1967: *Biochem. J.*, 103: 49-54.
 Clark, A. G.; Murphy, S. L.; Smith, J. N., 1969: *Biochem. J.*, 113: 89.
 Hook, G. E. R.; Smith, J. N., 1967: *Biochem. J.*, 102: 504-10.
 Kallman, B. J.; Andrews, A. K., 1963: *Science*, 141: 1050-1.
 Lipke, H.; Kearns, C. W., 1960: *Adv. Pest Control Res.*, 3: 253.
 Nakatsugawa, T.; Dahm, P. A., 1965: *J. Econ. Entomol.*, 58: 500-9.
 Oppenoorth, F. J., 1965: *Meded. Landbouwhougesch. Gent.*, 30: 1390.
 Perrott, D. C. F.; Brewerton, H. V.; McGrath, H. J. W., 1966: *N.Z. J. Sci.*, 9: 145-60.
 Peterson, J. E.; Robinson, W. H., 1964: *Toxicol. Appl. Pharmacol.*, 6: 312-7.
 Tsukamoto, M., 1961: *Botyu-Kagaku*, 26: 74-87.
 Williams, R. T., 1959: *Detoxication Mechanisms*, 2nd ed. Chapman & Hall, London.