The Role of Nonoxidative Metabolism in Organophosphorus Resistance

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The role of nonoxidative metabolism in organophosphorus resistance is discussed. Carboxylesterases have been partially purified from resistant and susceptible strains of certain insect and mite species. Comparative studies suggest interspecific differences in the nature of the enzymes. Although phosphatases appear to be important in resistance mechanisms, the significance of their contribution has not been well assessed. Contradictory reports with regard to substrate specificity of phosphatases are referred to. Glutathione transferases have recently been demonstrated to be another mechanism of organophosphorus resistance. In some cases the enzymes not only dealkylate but also dearylate organophosphorus substrates by conjugation with glutathione. The presence of either high or low nonspecific esterase activity has also been found to be associated with organophosphorus resistance. The possible involvement of nonspecific esterases in degradation is conjectured.

In the early 1950's, the resistance to organophosphorus insecticides was first noticed after failure to control certain pest species in the field. Subsequently, these results were reproduced in the laboratory under controlled conditions. The development of resistance in wild populations by selection proved that resistance was a preadaptive phenomenon (for a review see Crow, 1957). Following the observation that resistance occurred, studies were undertaken in many laboratories to try and identify the resistance mechanisms. At least three major factors have so far been recognized as contributing to organophosphorus resistance, *i.e.*, decrease in cuticular permeability, increase in detoxication, and modification of the target enzyme, cholinesterase.

In the present review only the aspects of detoxication will be discussed, with special emphasis on the role that nonoxidative metabolism plays in organophosphorus resistance. The metabolism of organophosphorus insecticides can be classified as those reactions involving the microsomal mixed-function oxidases and those confined to reactions generally associated with the soluble fraction. While the former has been receiving a great deal of attention from many workers and deserves special consideration, the latter appears also to play an important role in organophosphorus resistance. Therefore, the present paper is devoted to summarizing the data available concerning nonoxidative metabolism as a factor in organophosphorus resistance. These reactions involve the carboxylesterases, the phosphatases, and the glutathione transferases. The possible role of nonspecific esterases in resistance will also be discussed.

CARBOXYLESTERASES

Carboxylesterases hydrolyze certain organophosphorus insecticides at the carboxyester linkage, resulting in the formation of the corresponding nontoxic acid (Figure 1). The enzyme has been studied extensively and has been accepted as the most important factor accounting for the selective toxicity of malathion between mammals and insects and between insect species and insect strains.

There are several papers which described the involvement of carboxylesterases in malathion resistance. Since the enzymes have been well discussed by previous reviewers (Dauterman, 1971; O'Brien, 1967), it will be referred to only briefly. Utilizing crude homogenate or purified enzymes, Matsumura and his colleagues demonstrated an interstrain difference in carboxylesterase activity with the mosquito, *Culex tarsalis* (Matsumura and Brown, 1961, 1963), the housefly, *Musca domestica* (Matsumura and Hogendijk, 1964a), and the two-spotted spider mite, *Tetranychus urticae* (Matsumura and Voss, 1964, 1965). They concluded that carboxylesterases of the two resistant insect species had the same affinity toward malathion as the corresponding susceptible strains (based on K_m measurements), indicating the interstrain difference was quantitative. On the other hand, the carboxylesterase in the resistant mite showed higher affinity than the susceptible one, suggesting a qualitative difference. Table I shows the substrate specificity of carboxylesterases of resistant and susceptible two-spotted spider mites. The 82.5-fold purified resistant enzyme exhibited higher activities against a malathion, parathion, and β-naphthyl benzoate, equal activity against malaoxon, and a lower activity against β-naphthyl acetate than the 48-fold purified susceptible enzyme. Although the carboxylesterase was suggested to be distinctly different from the phosphatase based on differences in DFP sensitivity, a great similarity between these enzymes, particularly between the carboxylesterase and the β-naphthyl benzoate hydrolyzing esterase, was noted.

Townsend and Busvine (1969) reported carboxylesterase involvement in malathion resistance in the blowfly, *Crysomya putoria*. Both the malathion carboxylesterase and the methyl propionate hydrolyzing esterase were located in the same subcellular fraction (*i.e.*, microsomes), the resistant strain showing higher activity against the former substrate but lower activity against the latter substrate rather than the susceptible strain (Table II). The close negative correlation of the malathion carboxylesterase to the methyl propionate hydrolyzing esterase was genetically associated with the level of resistance.

In conclusion, the involvement of carboxylesterases in malathion resistance has been well established. However, it is interesting to note that the properties of carboxylesterases in the resistant strains seem to vary considerably according to the insect species. For instance, the enzyme in the mosquito was mainly located in the mitochondrial fraction, whereas that in the blowfly was found in the microsomal fraction. Furthermore, the higher carboxylesterase activity in the resistance mosquito was closely associated with low β-naphthyl benzoate hydrolysis and the opposite was true in the case of the two-spotted spider mite.

PHOSPHATASES

Hydrolytic reactions which cleave off the leaving groups of organophosphorus insecticides and result in the nontoxic dialkyl phosphorothioic or phosphoric acids have been well documented (Dauterman, 1971) (Figure 2). Various names have been used to describe the enzymes which catalyze these reactions; however, a general term "phosphatase" will be used in the present discussion.

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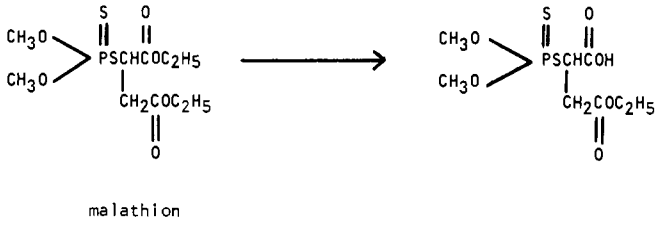


malathion

Figure 1. Malathion hydrolysis by carboxylesterase (Matsumura and Brown, 1963; Matsumura and Hogendijk, 1964a; Matsumura and Voss, 1965; Townsend and Busvine, 1969).

Table I. Substrate Specificity of Partially Purified Carboxylesterase[a] of Resistant (R) and Susceptible (S) Two-Spotted Spider Mites, *Tetranychus urticae*[c]

Substrate	Activity[b] S	Activity[b] R
Malathion		
Carboxyesteratic	4.42×10^{-4}	7.56×10^{-4}
Phosphoroesteratic	5.15×10^{-4}	7.40×10^{-4}
Malaoxon	0.4×10^{-4}	0.4×10^{-4}
Parathion	4.18×10^{-4}	5.22×10^{-4}
β-Naphthyl acetate	6.55×10^{-1}	3.4×10^{-1}
β-Naphthyl benzoate	2.65×10^{-2}	5.00×10^{-2}
o-Nitrophenyl acetate	3.52×10^{-2}	2.56×10^{-2}

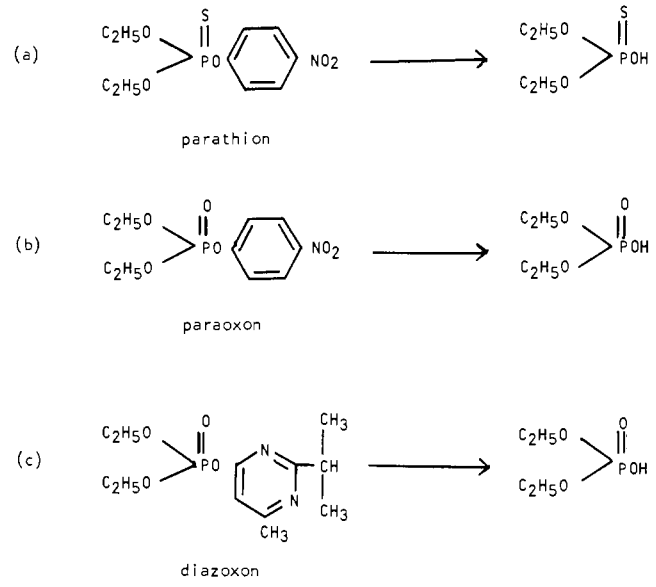
[a] 82.5-fold for R and 48-fold for S. [b] μmole/ml/30 min. [c] Data from Matsumura and Voss (1965).

Table II. Subcellular Distribution of Carboxylesterase and Nonspecific Esterase Activities in Resistant (R) and Susceptible (S) Blowflies, *Crysomya putoria*[c]

Subcellular fraction	Malathion[a] S	Malathion[a] R	Methyl propionate[b] S	Methyl propionate[b] R
Homogenate	1.2	16.2	8.2	0.9
10,000 × g supernatant	2.0	12.2	7.5	0.7
100,000 × g precipitate	1.4	12.0	7.3	0.7
100,000 × g supernatant	0	0.02	0.6	0.1

[a] μg of malathion monoacid produced/fly/30 min. [b] μmole of H^+ produced/fly/30 min. [c] Data from Townsend and Busvine (1969).

The close relationship of phosphatases with organophosphorus resistance has long been conjectured, since van Asperen and Oppenoorth (1959) observed unusually low esterase activity in resistant houseflies. However, the contribution of the enzyme to resistance mechanisms was not well established owing to methodological deficiencies in the early studies. Recent studies on the metabolism of organophosphorus insecticides have shown that a common metabolite can be derived by a number of different processes; thus the dialkyl phosphorothioic or phosphoric acids, which were originally considered to be phosphatase products, can be formed by the mixed-function oxidases as well as by glutathione transferases. Therefore early reports based solely on the nature of products cannot be accepted as proof for a particular enzyme system, as was pointed out by Oppenoorth (1971). In order to distinguish one enzyme system from another with regard to the resistance mechanism, it is necessary to examine, at least, the subcellular localization of the enzyme, cofactor requirements, the nature of the products, and the response to specific inhibitors. Despite a great deal of speculation and much effort with regard to the phosphatase involvement in organophosphorus resistance mechanisms, only a few papers are available which clearly distinguish this enzyme system from others.



parathion

paraoxon

diazoxon

Figure 2. Phosphatase hydrolysis in OP resistant insects. (a) Matsumura and Hogendijk (1964b). (b) Welling *et al.* (1971). (c) Lewis and Sawicki (1971).

Table III. Paraoxon Degradation by Homogenates of Resistant (E_1) and Susceptible (E_2) Houseflies, *Musca domestica*[b]

Strain	Paraoxon concentration, M	Paraoxon degraded[a]
E_1	1.8×10^{-5}	216 ± 17
	3.6×10^{-6}	152 ± 13
		Diethyl phosphate formed[a]
	3.2×10^{-6}	130
E_1	2.7×10^{-6}	121
	2.7×10^{-6}	147
	2.7×10^{-6}	22
E_2	2.7×10^{-6}	10

[a] μμmole/fly/hr. [b] Data from Welling *et all* (1971).

Matsumura and Hogendijk (1964b) compared the phosphatase activity in resistant and susceptible strains of the housefly. The partially purified enzyme from the resistant strain showed higher activity against parathion than that of the susceptible strain and produced diethyl phosphorothioic acid.

Welling *et al.* (1971) investigated the *in vitro* degradation of paraoxon in resistant and susceptible strains of the housefly. The microsomal fraction of the resistant strain degraded paraoxon and produced diethyl phosphoric acid as the major product, as well as two other minor products. The activity was inhibited by *n*-propyl paraoxon and *p*-chloromercuribenzoate but not by Sesamex, indicating the reaction was not catalyzed by the mixed-function oxidases. The results would indicate that the enzyme responsible for this reaction was phosphatase. It was also shown that production of diethyl phosphoric acid from paraoxon by whole-fly homogenates was remarkably higher in the resistant strain (E_1) than in the susceptible strain (E_2) (Table III). The data were interpreted as an indication that the resistant flies possess higher phosphatase activity than the susceptible flies, and this difference was responsible for the resistance mechanism. These results were also cited by Oppenoorth (1971) as strong evidence to support the "mutant aliesterase" hypothesis, which will be discussed later. However, as was mentioned previously, one cannot exclude the effect of other enzyme systems on the degradation, since Welling *et al.* (1971) used whole-fly homogenates instead of isolated microsomes for the interstrain comparison of activity. Whole-fly homogenates con-

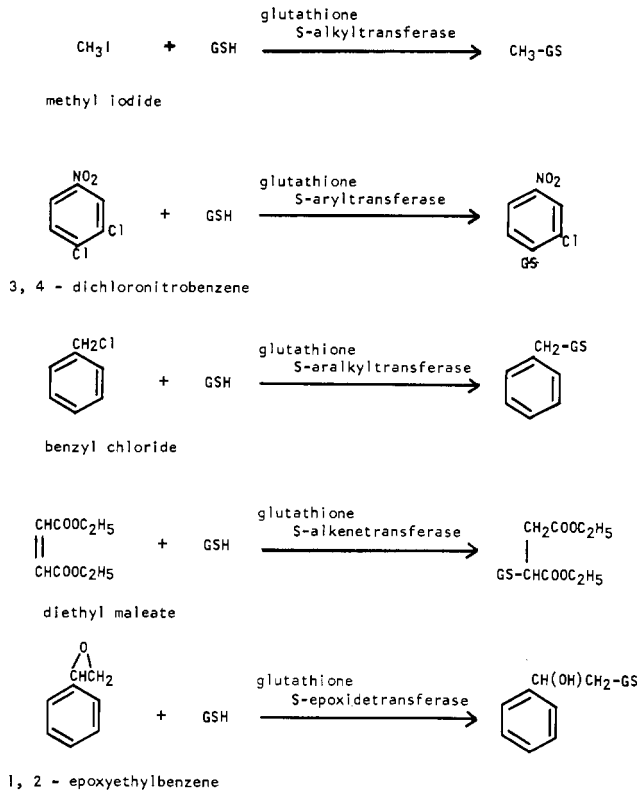


Figure 3. General transferase reactions with glutathione (Boyland and Chasseaud, 1969).

tain endogenous NADPH and GSH which would enhance the mixed-function oxidases and glutathione transferase activity, respectively.

Lewis and Sawicki (1971) reported that microsomes from resistant and susceptible houseflies produced diethyl phosphoric acid from diazoxon and paraoxon in the absence of air or NADPH, and showed some interstrain difference in activity. Based on this finding, they suggested that the phosphatase was involved in the resistance mechanism.

It may be appropriate to refer here to an argument on whether a phosphatase in resistant houseflies is active against both phosphorothioate and phosphate insecticides or only against the latter. In the above-mentioned paper, Matsumura and Hogendijk (1964b) used [^{32}P]parathion as the substrate for the measurement of phosphatase activity. Since a partially purified enzyme preparation was used, it seems improbable that the mixed-function oxidases were involved in the reaction. However, according to Welling *et al.* (1971) and Nakatsugawa *et al.* (1969), they could not reproduce the results with [^{14}C]parathion or [^{35}S]parathion. Thus most of the recent reviewers (Dahm, 1969; Dauterman, 1971; Oppenoorth, 1971; Wilkinson, 1971) concluded that phosphates are preferred substrates for hydrolysis by phosphatase, but phosphorothioates probably are not.

However, there are some other studies which indicated that phosphorothioates are hydrolyzed by phosphatases. For instance, Kojima and O'Brien (1968) claimed that the washed mitochondrial and soluble fractions from rat liver homogenates which produced diethyl phosphoric acid from paraoxon, without the addition of cofactors, were also active but to a lesser extent in the degradation of parathion. Matsumura and Sakai (1968) also reported that five esterase bands separated by means of the thin agar layer electrophoresis from the postmitochondrial fraction of the American cockroach, *Periplaneta americana*, demonstrated degradative activity against parathion and diazinon. It seems that further studies are required to clarify the substrate specificity of phosphatases in resistant houseflies with respect to phosphorothioate and phosphate insecticides and its variation among insect species as well as mammals.

In conclusion, although it appears certain that phosphatases are important in the resistance mechanism, it can be said that the significance of their contribution to resistance has not been well assessed.

GLUTATHIONE TRANSFERASES

Certain foreign compounds administered to mammals are excreted as mercapturic acid derivatives. The mechanism of the mercapturic acid formation involves initial conjugation of the foreign compound with glutathione (Boyland, 1962). This reaction is catalyzed by a group of glutathione transferases which have been classified into glutathione *S*-alkyltransferase, *S*-aryltransferase, *S*-aralkyltransferase, *S*-alkenetransferase, and *S*-epoxidetransferase (Boyland and Chasseaud, 1969). Figure 3 shows each reaction with model substrates. It has been suggested that these enzymes are distinctive based on differences in response to pH change, heat stability, and distribution in different organs.

The involvement of glutathione transferases in the metabolism of organophosphorus insecticides has recently been reviewed by several workers (Dauterman, 1971; Eto and Ohkawa, 1969; Hollingworth, 1969). Information obtained with methyl parathion, methyl paraoxon, fenitrothion, mevinphos, tetrachlorvinphos, bromophos, and azinphosmethyl indicated that the O-dealkylation of organophosphorus insecticides was an important degradative reaction. These studies demonstrated that mammalian livers, as well as some other organisms, contain enzymes responsible for this reaction. The enzyme responsible seems to be glutathione *S*-alkyltransferase, since the activity was located in the soluble fraction of the homogenates, required GSH, exhibited preference for methyl groups, and was inhibited by methyl iodide. On the other hand, Shishido *et al.* (1972) demonstrated that the rat liver and the fat body of American cockroaches contained enzymes active in the conjugation of the pyrimidine moiety of diazinon with GSH. The enzyme system was active against various *O,O*-dialkyl-substituted diazinon and diazoxon analogs. At present it is not known whether the enzyme which catalyzes the dearylation reaction of diazinon is identical to or different from the above-mentioned enzymes which catalyze the O-dealkylation reaction of other organophosphorus compounds.

Only recently have glutathione transferases been implicated in organophosphorus resistance (Figure 4). Lewis (1969) first reported that several diazinon-resistant strains of the housefly, which were known to have the gene "a" for low aliesterase activity, showed higher activities in the O-deethylation of diazinon and diazoxon in the presence of GSH than the other resistant and susceptible strains tested. Lewis and Sawicki (1971) confirmed that the housefly-resistant strains, which inherit factors for low aliesterase and therefore high phosphatase activities on chromosome II, also possessed high glutathione transferase activities. The soluble enzymes produced not only desethyl diazinon from diazinon or desethyl diazoxon from diazoxon but also diethyl phosphorothioic acid or diethyl phosphoric acid, respectively, in the presence of GSH. This finding would indicate that both O-dealkylation and dearylation occurred, although the actual conjugation of the pyrimidine moiety with GSH was not detected because ethoxy- or phosphorus-labeled substrates were employed. The formation of desethyl diazinon or desethyl diazoxon was much greater than that of the dearylation products in this system. In the same paper, an unusual observation was also made that GSH stimulated the NADPH-dependent system of the microsomes, although the mechanism of this stimulation was not clarified.

Oppenoorth *et al.* (1972) compared the glutathione-dependent degradation of parathion by the soluble fraction

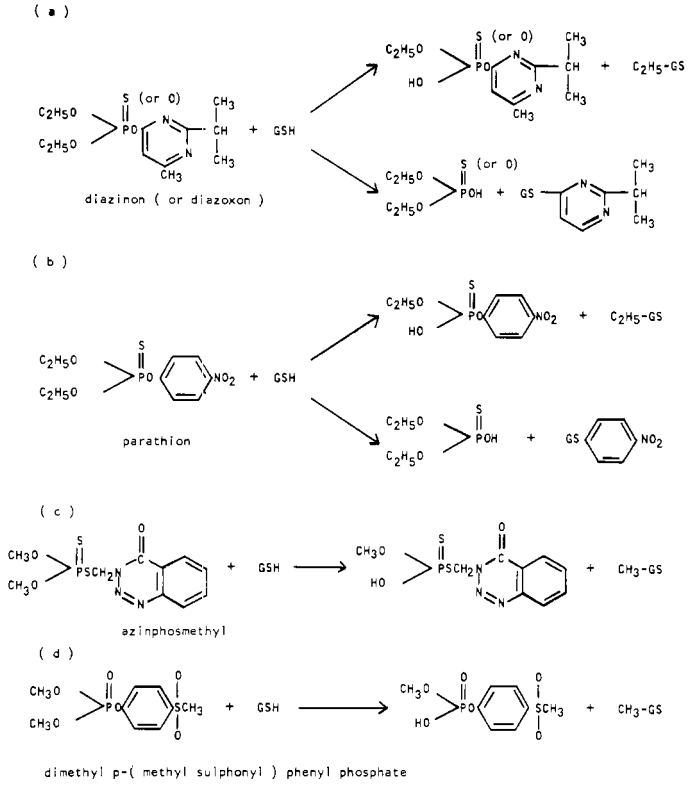


Figure 4. Transferase reactions with organophosphates in OP resistant species. (a) Lewis and Sawicki (1971). (b) Oppenoorth *et al.* (1972). (c) Motoyama *et al.* (1971); Motoyama and Dauterman (1972). (d) Bull and Whitten (1972).

from several resistant and susceptible housefly strains. Utilizing ethoxy-^{14}C-labeled parathion, three products were detected, *i.e.*, diethyl phosphorothioic acid, desethyl parathion, and ethyl glutathione, indicating again that both O-dealkylation and dearylation occurred (Table IV). However, in contrast to the previous report with diazinon (Lewis and Sawicki, 1971), the production of diethyl phosphorothioic acid was much higher than that of the O-dealkylated product. Since the resistant strain, having a normal aliesterase level, as well as the strains having a low aliesterase activity, exhibited higher parathion degradation by this system, the authors suggested that the gene "g" for glutathione transferase activity was not identical with the gene "a" for low aliesterase activity, although both were found to be located on the same chromosome II (Table V). The contribution of the glutathione transferase to parathion resistance was, however, concluded to be of minor importance because the level of activity was not proportional to the level of resistance.

Yang *et al.* (1971) observed faster degradation of diazinon by the soluble fraction of a multiresistant housefly strain in the presence of GSH, and detected diethyl phosphorothioic acid, diethyl phosphoric acid, and some other products. Using the same housefly strains, Motoyama and Dauterman (1972) observed a significant interstrain difference in the degradation of azinphosmethyl by the soluble fractions in the presence of GSH (Figure 5). With methoxy-^{14}C- or phosphorus-labeled substrates, the only metabolites detected were desmethyl azinphosmethyl and/or methyl glutathione. The fact that no dimethyl phosphorothioic acid was found may indicate that the benzazimide moiety was not a suitable substrate for the dearylation reaction by the soluble fraction. It appears that the glutathione *S*-alkyltransferase plays a fairly important role in the resistance mechanism in this housefly strain, because substitution of the dimethoxy group of azinphosmethyl by diethoxy group resulted in a marked decrease in the resistance level, *i.e.*, from over 1600- to 25-fold. When the enzyme(s) was incubated with methyl iodide and 3,4-dichloronitrobenzene, which are known substrates for glutathione *S*-alkyltransferases and *S*-aryltransferases, respectively, the resistant strain again demonstrated higher activities than the susceptible strain (Figure 5). Gel filtration of Sephadex G-100 and G-200, as well as ion-exchange chromatography on DEAE-Sephadex A-50 and

Table IV. Relative Amount of Parathion Metabolites by Glutathione-Dependent Soluble Enzymes in Houseflies, *Musca domestica*[a]

Strain	%		
	$(C_2H_5O)_2P(S)OH$	Desethyl parathion	C_2H_5GS
G	73	12	15
Nic	61	22	17
29	44	26	30

[a] Data from Oppenoorth *et al.* (1972).

Table V. Parathion Degradation by Glutathione-Dependent Soluble Enzymes in Resistant and Susceptible Houseflies, *Musca domestica*[b]

Strain	Other factors known for resistance	Nonspecific esterase activity	Degradation[a]
acr	(Susceptible)	High	0.01
Fc	MFO	High	0.13
29	Glutathione transferase	Low	0.22
E_1	Phosphatase	Low	0.25
Nic	MFO	High	0.36
G	Carboxylesterase	Low	1.47

[a] μg of parathion/0.5 abdomen/hr. [b] Data from Oppenoorth *et al.* (1972).

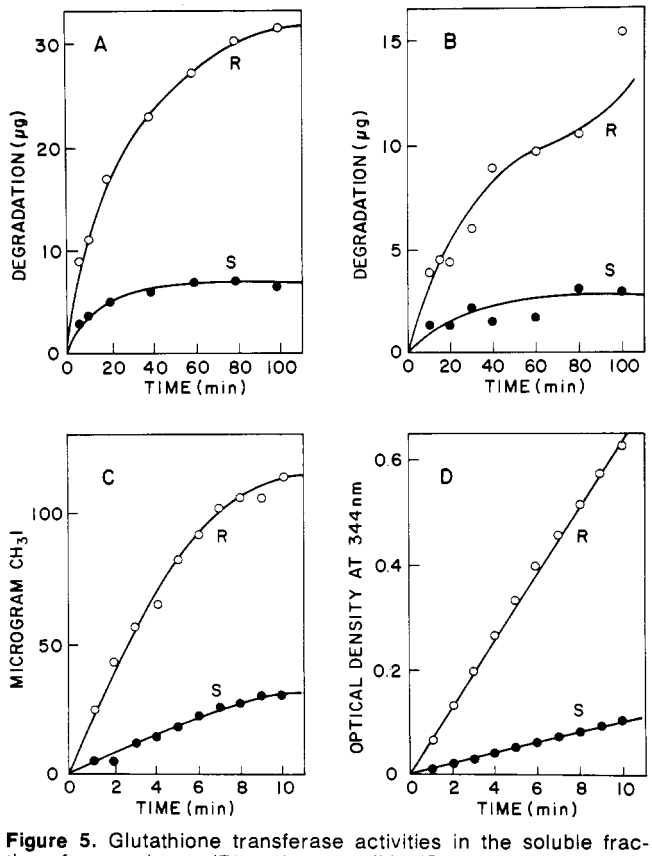


Figure 5. Glutathione transferase activities in the soluble fractions from resistant (R) and susceptible (S) houseflies. A, azinphosmethyl; B, diazinon; C, methyl iodide; D, 3,4-dichloronitrobenzene. A, C, and D: Motoyama and Dauterman (1972). B: Motoyama and Dauterman, unpublished data.

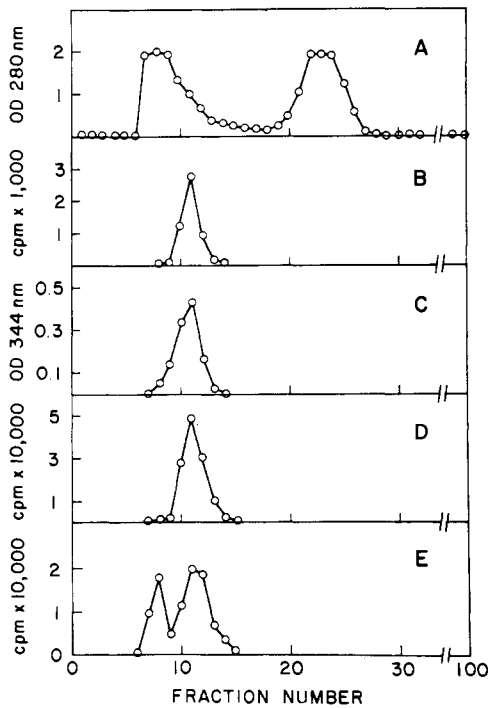


Figure 6. Sephadex G-100 chromatography of glutathione-dependent soluble enzymes from resistant houseflies. A, protein level; B, methyl iodide; C, 3,4-dichloronitrobenzene; D, azinphosmethyl; E, diazinon. A, B, C, and D: Motoyama and Dauterman (1972). E, Motoyama and Dauterman, unpublished data.

QAE-Sephadex A-50 of the soluble enzymes, failed to separate the glutathione transferase activities for the substrates azinphosmethyl, diazinon, methyl iodide, and 3,4-dichloronitrobenzene (Figure 6). Recent studies in our laboratory with some insecticidal and noninsecticidal substrates suggest that the glutathione-dependent soluble enzymes in resistant houseflies possess high activities of DDT dehydrochlorination, *r*-BHC degradation, conjugation of benzyl chloride, and diethyl maleate, which are known as glutathione *S*-aralkyltransferase and *S*-alkenetransferase substrates, respectively. However, the same level of 1,2-epoxyethylbenzene conjugation, a glutathione *S*-epoxidetransferase substrate, was observed in both the resistant and susceptible strain of houseflies. At the present time it is not clear whether this multiresistant housefly strain has several different glutathione transferases of high activity or one multiactive enzyme is present. The information obtained with several different chromatographic systems indicates that these enzymes, if there are more than one, have very similar molecular weights as well as molecular charges. Further work is in progress on this enzyme(s) with regard to its purification and characterization.

The housefly is not the only case in which the involvement of glutathione transferases has been reported. Motoyama *et al.* (1971), studying the mechanism of azinphosmethyl resistance in the predacious mite, *Neoseiulus fallacis*, found higher degradative activity in the resistant strain (Table VI). The activity was in the soluble fraction of the homogenate, enhanced by the addition of GSH, and the major metabolite found with the phosphorus-labeled compound was desmethyl azinphosmethyl. Since no differences were observed in all the other factors investigated, *i.e.*, cholinesterase sensitivity and cuticular permeability, the resistance mechanism was attributed to the difference in glutathione *S*-alkyltransferase activity. The substitution of the dimethoxy group of azinphosmethyl by other dialkoxy groups decreased the resistance level significantly, supporting this conclusion.

The involvement of glutathione transferases was also reported with the tobacco budworm larvae, *Heliothis virescens*, by Bull and Whitten (1972). The rate of *in vitro* demethylation of *O,O*-dimethyl *p*-(methylsulfonyl)phenyl phosphate was enhanced by the addition of GSH, the resistant strain showing higher activity than the susceptible strain. We also found (unpublished data) that the soluble fraction of the resistant tobacco budworms had higher activity in azinphosmethyl degradation than the susceptible larvae in the presence of GSH.

Thus it now appears that glutathione transferases are another important mechanism for organophosphorus resistance. The degree of contribution of the enzymes to resistance may vary, depending upon the insecticidal substrates involved and the nature of the enzymes. However, little is known concerning the nature of these enzymes in resistant species and this area is a fruitful field for further investigations.

Table VI. Effect of Cofactors on Azinphosmethyl Degradation by Homogenates of Resistant (R) and Susceptible (S) Predacious Mites, *Neoseiulus fallacis*[b]

Cofactor	Degradation[a] S	R
None	0	6.0
GSH	10.8	61.2
NADPH	9.8	17.2
GSH + NADPH	25.0	75.8

[a] μg of azinphosmethyl equivalents/100 mg of mites/2 hr. [b] Data from Motoyama *et al.* (1971).

Table VII. Relationship between Nonspecific Esterase Activity and OP Resistance

Species	Substrate	Ref
I. OP resistance associated with low esterase activity		
Musca domestica	Methyl *n*-butyrate	*a, b*
Culex tarsalis	β-Naphthyl benzoate	*c*
Chrysomya putoria	Methyl propionate	*d*
Tetranychus urticae	β-Naphthyl acetate	*e*
II. OP resistance associated with high esterase activity		
Neophotettix cincticeps	β-Naphthyl acetate	*f, g*
Laodelphax striatellus	β-Naphthyl acetate	*h*
Myzus persicae	α-Naphthyl acetate	*i*
Culex pipiens fatigans	α- and β-naphthyl acetate	*j*
Culex pipiens pallens	β-Naphthyl acetate	*k*
Culex tarsalis	α-Naphthyl acetate	*c*
Neoseiulus fallacis	α-Naphthyl acetate	*l*
Tetranychus urticae	β-Naphthyl benzoate	*e*
Panonychus citri	β-Naphthyl acetate	*m*

[a] van Asperen and Oppenoorth (1959). [b] Bigley and Plapp (1960). [c] Matsumura and Brown (1963). [d] Townsend and Busvine (1969). [e] Matsumura and Voss (1965). [f] Kasai and Ogita (1965). [g] Ozaki *et al.* (1966). [h] Ozaki and Kasai (1970). [i] Needham and Sawicki (1971). [j] Stone and Brown (1969). [k] Yasutomi (1970). [l] Motoyama *et al.* (1971). [m] Motoyama (1968).

POSSIBLE ROLES OF NONSPECIFIC ESTERASES

The term "nonspecific esterases" will be used in the following discussion to refer to enzymes which have been detected with certain aliphatic or aromatic esters as substrates, but their enzymological identity and function with regard to resistance have not been established.

A relationship between organophosphorus resistance and nonspecific esterases has been reported many times from two distinct opposite observations, *i.e.*, resistance associated with low nonspecific esterase activity and resistance associated with high nonspecific esterase activity (Table VII). The former phenomenon resulted in the famous "mutant aliesterase" hypothesis (Oppenoorth and

van Asperen, 1960). This hypothesis proposes mutation of the gene "a" which is responsible for high aliesterase activity in wild-type housefly population to a detoxication enzyme, a phosphatase, and suggests that this increase in the ability to detoxify organophosphorus compounds is responsible for the resistance. Much work has been done to prove this hypothesis. Since O'Brien (1967) has already reviewed the work in detail, it will be discussed here only briefly. Oppenoorth (1971) answered questions raised by O'Brien (1967) and proclaimed the validity of the hypothesis, although to a lesser extent than originally proposed. It appears that the hypothesis has two different aspects which should be evaluated separately.

The first part of the hypothesis is concerned with the inseparable relationship between low esterase levels with organophosphorus resistance and/or high degradative activity in certain housefly strains. Georghiou (1965) reviewed this aspect and concluded that the hypothesis does not cover all cases because various strains of houseflies have been found showing contradiction to the above observation, *i.e.*, those which have low esterase activity but are organophosphorus susceptible, those which have high esterase activity and are organophosphorus resistant, or cases which show no correlation between esterase activity and organophosphorus resistance. Therefore, the first part of the hypothesis, in order to exclude the possibility of coincidence due to close linkage, needs more direct proof which shows the identity of both enzymes detected with organophosphorus and nonspecific esterase substrates. This would require purification of the phosphatase, which has low esterase activity from the resistant houseflies, and its counterpart, which has high esterase activity from the susceptible houseflies. Such an enzyme is not rare, as was mentioned above for the carboxylesterase and β-naphthyl acetate hydrolyzing activity in the two-spotted spider mite (Matsumura and Voss, 1965). A similar negative correlation was also cited previously with the blowfly with regard to the carboxylesterase and methyl propionate hydrolyzing activity (Townsend and Busvine, 1969). As for the second part of the hypothesis, it is now apparent that increased phosphatase activity alone does not explain all the mechanisms of resistance in the housefly. Resistant housefly strains, which have low aliesterase activity, also possess higher activities in the mixed-function oxidases and/or glutathione transferases (Lewis, 1969; Lewis and Sawicki, 1971; Oppenoorth *et al.*, 1972). The degree of increased phosphatase activity to the resistance mechanism should be determined by comparing the efficacy of each system *in vivo*.

There are a number of reports which demonstrate a correlation between high nonspecific esterase activity and organophosphorus resistance.

Ozaki and Kasai (1970) reported that the multiorganophosphorus resistant strain of the brown planthopper, *Laodelphax striatellus*, exhibited higher activity for β-naphthyl acetate hydrolysis than the susceptible strain and it was due to the presence of an extra electrophoretic band. The genetic studies suggested that the esterase activity of the electrophoretic band and malathion resistance depended on the same factor (Table VIII).

An organophosphorus-resistant strain of the predacious mite, whose resistance mechanism was suggested to be due to glutathione transferases, demonstrated higher activity for α-naphthyl acetate hydrolysis than the susceptible strain (Motoyama *et al.*, 1971) (Table IX). Electrophoresis revealed the presence of two extra esterase bands in the resistance strain. The activity was located in the soluble fraction of the homogenate (Table X). Similar phenomena have been reported with the green rice leafhopper, *Neophotettix cincticeps*, for malathion resistance (Kasai and Ogita, 1965; Ozaki *et al.*, 1966), the mosquito, *Culex pipens pallens*, for diazinon resistance (Yasutomi, 1970), another mosquito, *Culex pipiens fatigans*, for fenthion resistance (Stone and Brown, 1969), the citus red mite, *Panonychus citri*, for dimethoate resistance (Motoyama, 1968), and the green peach aphid, *Myzus persicae*, for general organophosphorus resistance (Needham and Sawicki, 1971).

Table VIII. Inheritance of Esterase Activity[a] and Response to Malathion in the Smaller Brown Planthopper, *Laodelphax striatellus*[c]

Strain	Frequency of E_7 band activity			LD_{50}[b]
	Low	Middle	High	
HE	0	0	140	11.17
LE	150	0	0	0.187
F_1, I	0	140	0	1.879
F_1, II	0	100	0	1.279
F_2	76	145	62	
Backcross, I	140	154	0	
Backcross, II	120	121	0	

[a] Substrate: β-naphthyl acetate. [b] μg of malathion/tube. [c] Data from Ozaki and Kasai (1970).

Table IX. The *in Vitro* Activity of Nonspecific Esterases of a Resistant (R) and a Susceptible (S) Strain of Predacious Mites, *Neoseiulus fallacis*[b]

Substrate concentration, M	Activity ± SD[a]	
	S	R
5×10^{-5}	1.12 ± 0.05	2.45 ± 0.12
1×10^{-4}	1.68 ± 0.08	4.07 ± 0.17
5×10^{-4}	2.96 ± 0.07	8.81 ± 0.31

[a] α-Naphthol, 10^{-2} μmol/3 ♀ mites/15 min. [b] Data from Motoyama *et al.* (1971).

Table X. Subcellular Distribution of Azinphosmethyl Degradation and α-Naphthyl Acetate Hydrolysis Activities in Resistant Predacious Mites, *Neoseiulus fallacis*[c]

Subcellular fraction	Azinphosmethyl degradation[a]	α-Naphthyl acetate hydrolysis[b]
Nuclei	0	0.20
Mitochondria	0	0.22
Microsomes	0	0.17
Soluble fraction	49.2	6.58

[a] μg of azinphosmethyl equivalents/100 mg of mites/2 hr in the presence of GSH. [b] μmole of α-naphthol/mg of mites/15 min. [c] Data from Motoyama *et al.* (1971).

The data reviewed here would indicate that the nonspecific esterases are definitely involved in the mechanisms of resistance in various insect and mite species. However, it is not clear which reactions with regard to the mechanism of resistance are catalyzed by the nonspecific esterases. In the case of azinphosmethyl resistance in the predacious mite, since the α-naphthyl acetate hydrolyzing activity was located in the same subcellular fraction as the glutathione transferase, attempts have been made in our laboratory to separate and identify both enzymes. Preliminary results indicate coincidence of both enzymes after Sephadex G-100 chromatography as well as DEAE-cellulose chromatography, indicating that both reactions are probably mediated by the same enzyme. In the case of malathion resistance in various species, the nonspecific esterases appear to be identical to carboxylesterases because there is some evidence which suggested that carboxylesterases have nonspecific esterase activities (Main and Braid, 1962) or nonspecific esterases have carboxylesterase activities (Matsumura and Sakai, 1968; Miyata and Matsumura, 1971; Sakai and Matsumura, 1968). In the case of fenthion resistance in the mosquito, *Culex pipiens*

fatigans (Stone and Brown, 1969), it is probable that a phosphatase was identical to the α- and β-naphthyl acetate hydrolyzing enzyme. This was suggested by the presence of an electrophoretic esterase band which hydrolyzes fenoxon, along with the fact that the largest interstrain difference in degradation was in the production of dimethyl phosphoric acid.

In conclusion, however, the definite identity of the nonspecific esterases with the detoxication enzymes in the organophosphorus resistant strains requires further proof.

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Third Generation Pesticides: the Potential for the Development of Resistance by Insects

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Certain strains of insects that show resistance to insecticides also show cross-resistance to insect juvenile hormone mimics or analogs. In some insects, juvenile hormone tolerance appears to be correlated with high levels of microsomal mixed-function oxidase activity. Genetic tests are described in which the inheritance of juvenile hormone cross-resistance in the housefly was measured. Cross-resistance was controlled by genetic factor(s) on chromosome II, the chromosome which controls high levels of oxidase activity. Experience with present insecticides suggests that through selective pressures from the use of the third generation insecticides high levels of resistance also may develop.

Insect juvenile hormones and compounds which mimic their effects have received a great deal of attention in the last several years as possible insect control agents. Insect hormone studies had their early beginning with Kopec (1922), who first suggested that insect molting was controlled by hormones. Later Wigglesworth (1934) showed that the molting process required a factor which appeared to come from the head of the insect. In 1936, Wigglesworth demonstrated that metamorphosis was inhibited by a factor from the corpora allata. During the intervening years, through the work of a great number of researchers, these early workers' findings have been confirmed.

In insects the processes of growth and development are controlled by three primary hormones, as shown diagrammatically in Figure 1. The brain hormone (BH) is produced by the neurosecretory cells of the brain, transported *via* axoms to the corpus cardiacum, and released from there. The brain hormone stimulates the release of a sec-

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