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Mechanism of Action of Organophosphorus and Carbamate Insecticides

by T. Roy Fukuto*

Organophosphorus and carbamate insecticides are toxic to insects and mammals by virtue of their ability to inactivate the enzyme acetylcholinesterase. This review addresses the mechanism of inhibition of acetylcholinesterase by organophosphorus and carbamate esters, focusing on structural requirements necessary for anticholinesterase activity. The inhibition of acetylcholinesterase by these compounds is discussed in terms of reactivity and steric effects. The role of metabolic activation or degradation in the overall intoxication process is also discussed.

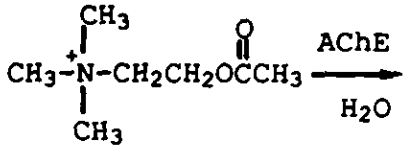
Introduction

The toxicity of insecticidally active organophosphorus and carbamate esters to animals is attributed to their ability to inhibit acetylcholinesterase (AChE, choline hydrolase, EC 3.1.1.7), which is a class of enzymes that catalyzes the hydrolysis of the neurotransmitting agent acetylcholine (ACh). The inhibition of AChE and related esterases, often referred to as serine hydrolases, has been clearly demonstrated to be the result of an actual chemical reaction between the enzyme and the organophosphate or carbamate ester (*1–3*). The phosphorylated or carbamylated enzyme is no longer capable of effecting the hydrolysis of ACh; this results in a buildup of the neurotransmitter at a nerve synapse or neuromuscular junction.

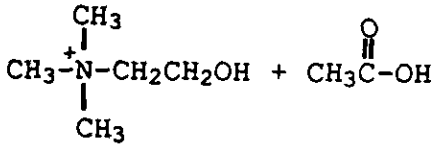
The organophosphorus and carbamate insecticides are represented by a wide variety of chemical structures having different chemical and physical properties. The toxicity of these materials to insects and mammals is determined by a number of factors that may affect the insecticides as they are absorbed, translocated to the target site, and as they inactivate the target, leading to poisoning. This review is concerned with the mode of action of organophosphorus and carbamate esters, with emphasis placed on structural requirements necessary for inhibition of AChE.

Acetylcholinesterase

Before entering into any discussion on the inhibition of AChE by organophosphorus and carbamate esters, it is appropriate first to provide a brief review of the enzyme. AChE is present and has been isolated from a wide range of animals, including mammals, birds, fish, reptiles, and insects (*4*). It is responsible for the rapid hydrolytic degradation of the neurotransmitter ACh into the inactive products choline and acetic acid, as indicated by Eq. (1). Acetylcholine is one of a number of



ACh (acetylcholine)



choline acetic acid (1)

physiologically important neurotransmitting agents and is involved in the transmission of nerve impulses to effector cells at cholinergic, synaptic, and neuromuscular junctions. The presence of AChE has been demonstrated in a variety of animal tissues and enzymes from a number of different sources, including fish electric organs, mammalian erythrocyte, insect and mammalian brain, and other tissues. These enzymes have been purified and characterized (*4–8*). AChE is virtually a ubiquitous enzyme in vertebrates and invertebrates, and in mammals, it is localized in certain areas of the central nervous system and in organs and glands that are controlled by the parasympathetic division of the autonomic nervous system.

The role of AChE in cholinergic transmission at a synaptic or cholinergic junction is depicted in the ele-

*Department of Entomology, University of California, Riverside, CA 92521.

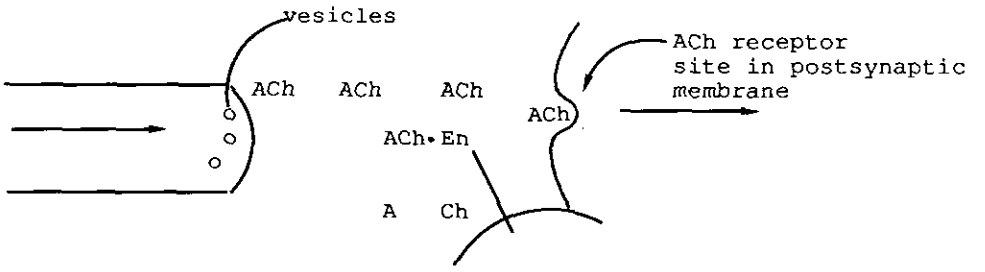


FIGURE 1. Scheme showing the role of AChE and ACh in cholinergic transmission.

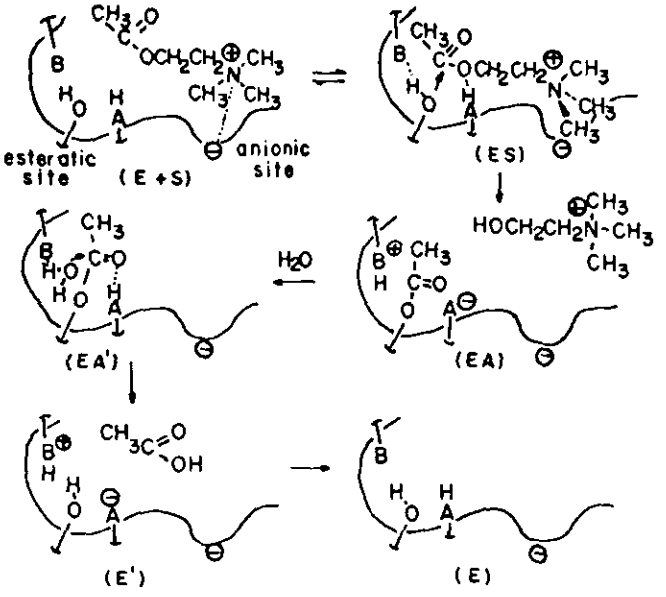


FIGURE 2. Mechanism of hydrolysis of acetylcholine by acetylcholinesterase (see text for details).

mentary scheme given in Figure 1. When a nerve impulse moves down a parasympathetic neuron and reaches a nerve ending, the ACh stored in vesicles in the ending is released into the synaptic or neuromuscular junction. Within 2 to 3 msec the released ACh interacts with the ACh receptor site on the postsynaptic membrane, causing stimulation of the nerve fiber or muscle. AChE serves as a regulating agent of nervous transmission by reducing the concentration of ACh in the junction through AChE catalyzed hydrolysis of ACh into choline (Ch) and acetic acid (A). These products do not stimulate the postsynaptic membrane. In the scheme En denotes the enzyme AChE; ACh · En— is the enzyme-substrate complex formed prior to hydrolysis of ACh into choline and acetic acid. When AChE is inactivated, e.g., by an organophosphorus or carbamate ester, the enzyme is no longer able to hydrolyze ACh; the concentration of ACh in the junction remains high, and continuous stimulation of the muscle or nerve fiber occurs, resulting eventually in exhaustion and tetany.

Based on a number of studies (*2*), a plausible mechanism for AChE-catalyzed hydrolysis of ACh is presented in Figure 2. ACh is drawn to the active site of the enzyme by electrostatic attraction between the positive charge on the ACh nitrogen atom and negative charge in the anionic site (structure E + S) resulting in the enzyme-substrate complex (ES). Acetylation of a serine hydroxyl (OH) in the esteratic site is catalyzed by the basic imidazole moiety B (histidine) and acidic moiety AH (tyrosine hydroxyl), leading to the acetylated enzyme EA. Deacetylation then takes place very fast, resulting in the free enzyme (E) within milliseconds. As presented, ACh hydrolysis by AChE has the elements of an acid-base catalyzed reaction, including both the acetylation and deacetylation reaction. The negative charge at the anionic site is attributed to the carboxylate anion of aspartic or glutamic acid. The reaction steps given in Figure 2 provide an elementary presentation of the AChE active site and a reasonable mechanism for the hydrolysis of ACh. The enzyme is in reality a highly complex protein, having in addition to the esteratic and anionic sites, a number of peripheral sites and hydrophobic areas (*5*).

While the preceding discussion has focused on AChE, it should be pointed out that there is at least one other type of cholinesterase enzyme beside AChE, namely pseudocholinesterase. AChE has the highest specificity for ACh of any other choline ester and pseudocholinesterase has the highest specificity for butyrylcholine. The physiological role of pseudocholinesterase in animals is not as well defined as that of AChE (*4*). However, both enzymes are inhibited by organophosphorus and carbamate esters.

Organophosphorus Insecticides

Mechanism of Inhibition

The inhibition of AChE by an organophosphorus ester (Fig. 3) takes place via a chemical reaction in which the serine hydroxyl moiety in the enzyme active site is phosphorylated in a manner analogous to the acetylation of AChE (Fig. 2, EA). In contrast to the acetylated enzyme, which rapidly breaks down to give acetic acid and the regenerated enzyme, the phosphorylated enzyme (Fig. 4) is highly stable, and in some cases, depending on the groups attached to the phosphorus atom (R and R′), it is irreversibly inhibited (*9*). The serine hydroxyl group, blocked by a phosphoryl moiety, is no longer able to participate in the hydrolysis of ACh. The inhi-

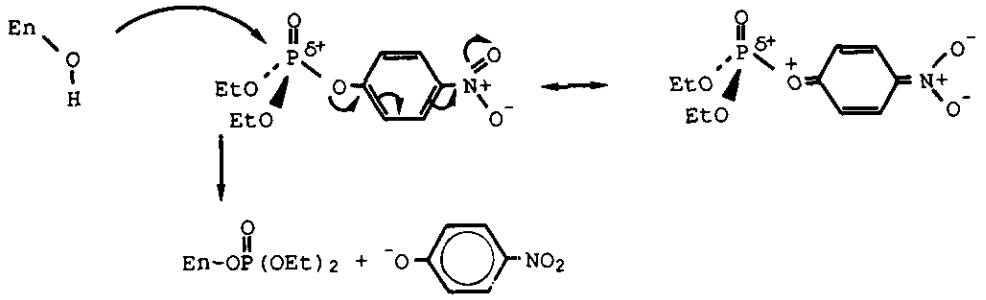
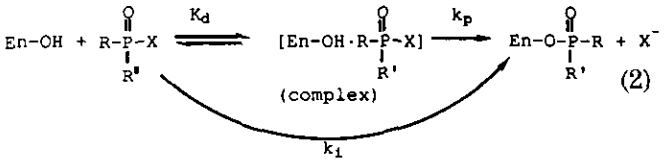


FIGURE 3. The effect of an electron-withdrawing substituent on the reactivity of paraoxon.

bition reaction takes place in a two-step process, as indicated by Eq. (2). In this equation



(2)

En—OH represents AChE in which the serine hydroxyl moiety (—OH) is emphasized, R and R′ are a variety of different groups (alkoxy, alkyl, amino, thioalkyl, etc.), X is the leaving group, K_d is the dissociation constant between the enzyme-inhibitor complex and reactants, k_p is the phosphorylation constant, and k_i is the bimolecular rate constant for inhibition and is equal to k_p/K_d (*10*). Since K_d provides a measure of the dissociation of the enzyme-inhibitor complex, it is regarded as an estimate for binding and is dependent on the structural and steric properties of the molecule. In contrast, the phosphorylation constant k_p is regarded as an estimate of the reactivity of the organophosphorus ester. The bimolecular rate constant k_i is dependent on the values of K_d and k_p and is generally regarded as the most useful parameter for the estimation of the inhibitory potency of an organophosphorus (and carbamate) anticholinesterase. According to Eq. (2), the moiety X is displaced from the phosphorus atom by the serine hydroxyl of the enzyme and is, therefore, referred to as the leaving group.

Reactivity and Steric Properties

Based on systematic investigations of the relationship between chemical structure and inhibition of AChE, it

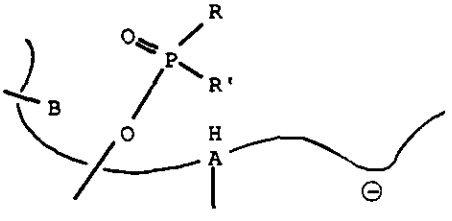


FIGURE 4. Phosphorylated, and irreversibly inhibited, AChE (see text and Figure 2 for details).

is apparent that the single most important property required in an organophosphate for anticholinesterase activity is chemical reactivity, i.e., the phosphorus ester must be reactive to the extent that the serine hydroxyl moiety in the enzyme is phosphorylated. Structure-activity studies have revealed a direct relationship between anticholinesterase activity and reactivity of the phosphorus atom. The inhibition of erythrocyte AChE by diethyl *p*-nitrophenyl phosphate (paraoxon) and some of its substituted phenyl analogs was found to proceed with pseudo first-order kinetics (*1*). The bimolecular rate constant for inhibition paralleled the rates of alkaline hydrolysis of these phosphates. It was subsequently demonstrated with a larger series of diethyl substituted phenyl phosphates that the inhibition of fly-head AChE by paraoxon analogs was related to the effect of the substituent on the liability of the P-O-phenyl bond as estimated by Hammett's sigma constants, shifts in P-O-phenyl infrared stretching frequencies, and hydrolysis rates (*11*). A plot of the log of anticholinesterase activity against Hammett's sigma constant (also P-O-phenyl stretching frequency) resulted in a good linear relationship with activity directly related to the electron-withdrawing properties of the substituents. The effect of an electron-withdrawing substituent on the reactivity of a diethyl substituted phenyl phosphate is demonstrated in Figure 4 with paraoxon. Because of the strong electron-withdrawing property of the nitro group, electrons are attracted away from the phosphorus atom, creating an electron-deficient center that facilitates a nucleophilic attack by the enzyme serine hydroxyl moiety on the phosphorus atom with simultaneous expulsion of the nitrophenoxide leaving group. Diethyl-substituted phenyl phosphates with weak electron-withdrawing or electron-donating substituents were weak inhibitors or were devoid of activity.

While chemical reactivity of the phosphorus atom is of prime importance for anticholinesterase activity, steric properties sometimes have a strong effect on the anticholinesterase activity of an organophosphorus ester. This became apparent from examining a series of ethyl *p*-nitrophenyl alkylphosphonates for anticholinesterase activity and alkaline hydrolysis rates where the alkyl group was varied (*12*). The rate of hydrolysis of the phosphonate ester to ethyl alkylphosphonic acid and *p*-nitrophenol, in general, decreased with an in-

crease in alkyl chain length, and it decreased strongly with branching in the 1 and 2 positions of the alkyl moiety. Determination of housefly-head anticholinesterase activities of these compounds revealed the relationship shown in Figure 5 between bimolecular rate constants for inhibition of housefly-head AChE (k_i) and pseudo first-order hydrolysis constants (k_{hyd}, pH 8.3). Although the plot between log k_i and log k_{hyd} showed a general trend toward linearity, close examination of the data revealed that many of the points followed a sigmoidal relationship, particularly those compounds with a straight chain alkyl. For example, as the chain length increased from three to six carbon atoms, the rate of inhibition of AChE dropped rapidly even though hydrolysis rates remained relatively constant. The plot reveals the influence of steric effects in the inhibition of AChE by these compounds, i.e., the inhibition rates are reduced as the alkyl moiety attached to the phosphorus atom becomes bulkier. The importance of steric effects in AChE inhibition was subsequently pointed out by Hansch and Deutsch (*13*) who showed that anticholinesterase activities for the compounds in Figure 5 were related to Taft's steric substituent constant, E_s, according to Eq. (3) where n is the number of compounds, s is the standard deviation, and r is the correlation coefficient.

$$\log k_i = 3.738E_s + 7.539$$
$$n = 13,\ s = 0.749,\ r = 0.901 \qquad (3)$$

While this study provided fundamental information on the effect of the P-alkyl moiety on the reactivity of alkylphosphonate esters, it also revealed that the P-alkyl moiety should be kept small (methyl or ethyl) in designing potential insecticides from phosphonate esters.

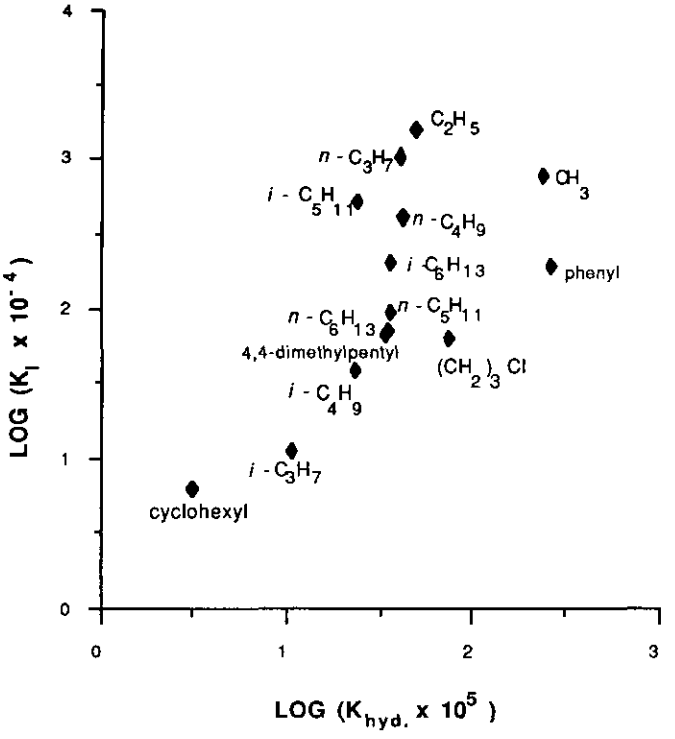


FIGURE 5. Relationship between log acetylcholinesterase inhibition constant k_i and log hydrolysis constant k_{hyd} for ethyl *p*-nitrophenyl alkylphosphonates. Data from Fukuto and Metcalf (*12*).

Structure of Organophosphorus Anticholinesterase Insecticides

Approximately 100 different organophosphorus insecticides have reached the stage of commercial development during the past four decades (*14*). Examination of these insecticides reveals a variety of different structures with virtually all of them falling within the general structure depicted in Figure 6, where R is methyl or ethyl; R′ is either methoxy, ethoxy, ethyl, phenyl, amino, substituted amino or alkylthio; and X is an appropriate leaving group. As indicated, the double-bonded oxygen may be replaced with sulfur. Examples of organophosphorus insecticides having each of the different R′ groups are given in Figure 7. Although R′ and RO for most of the organophosphorus insecticides are the same, i.e., R′=RO=CH_3O or C_2H_5O, as in the case of methyl parathion and diazinon, the examples given previously indicate the range of groups found in R′. Compounds where R′=RO and R is either propyl or isopropyl are less effective insecticides compared to those where R is methyl or ethyl and, therefore, they have generally not been developed as insecticides. Since the previous examples are all effective insecticides (and nematicide in the case of nemacur), it may be assumed that each compound or a corresponding metabolic product is able to phosphorylate AChE to give the phosphorylated enzyme as indicated in Figure 6, where R and R′ are the same as in the examples in Figure 7, or R′ is converted metabolically into another functional group [for example the acetamido moiety in acephate to amino in methamidophos (*15*)].

The leaving group is represented by a much broader range of structures, and the largest number of variations in the structure of an organophosphorus insecticide is found in X. The ability of X to leave the phosphorus atom is governed by the chemical nature of X and that of the other groups attached to phosphorus. For organophosphorus esters of high insecticidal activity, X is an electronegative group or a group containing an electronegative substituent leading to a compound with a reactive or labile P—X bond. In some instances

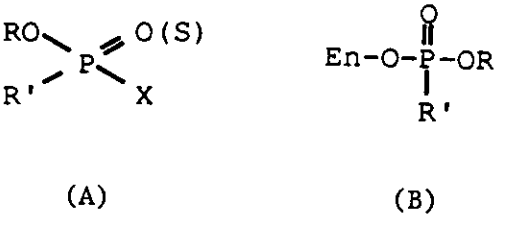


FIGURE 6. General structure of organophosphorus insecticides (*A*) and phosphorylated AChE (*B*). R is methyl or ethyl; R′ is either methoxy, ethoxy, ethyl, phenyl, amino, substituted amino or aklylthio: X is an appropriate leaving group and En is AChE.

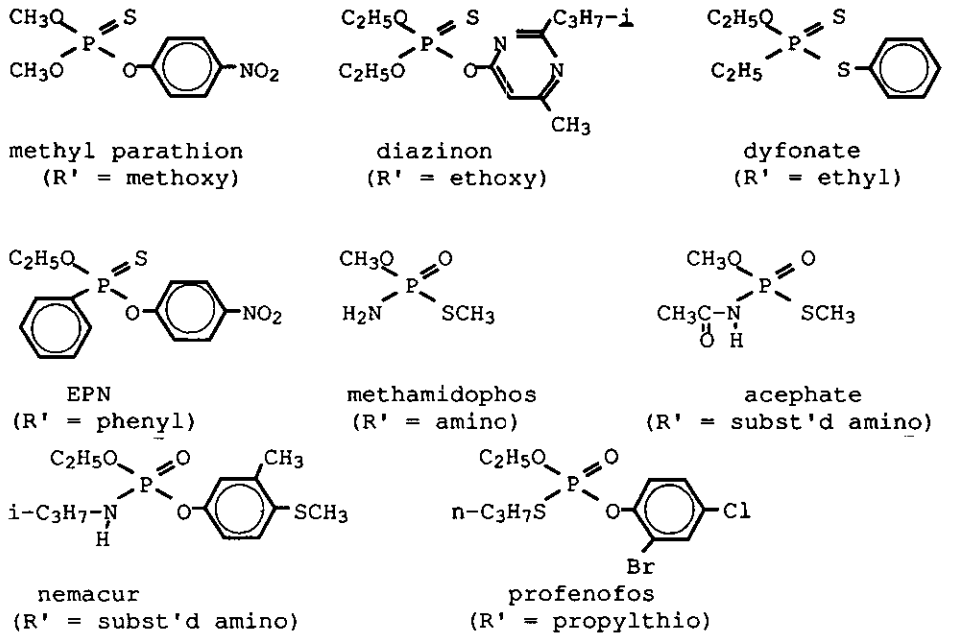


FIGURE 7. Different organophosphorus compounds showing variation in R'.

X is a moiety that is metabolically activated to give a labile P—X bond. In most cases X is a substituted phenoxy or aromatic group containing hetero atoms, substituted thioalkyl, or substituted alkoxy. Additional examples of prominent organophosphorus insecticides with variation in the leaving group, X, are given in Figure 8. The large number of organophorus insecticides that have attained commercial importance is attributable primarily to the large number of leaving groups possible. In no other class of insecticides has it been possible to have broader variation in structure and in spectrum of insecticidal activity.

Metabolic Activation

More than half of the compounds in Figures 7 and 8 contain the P=S moiety. Phosphorothionate esters (P=S) are generally poor anticholinesterases, yet all of the compounds given in Figures 7 and 8 are potent insecticides. The poor anticholinesterase activity of P=S esters is explained on the basis of their relatively low reactivity, attributed to the smaller extent to which the P=S bond is polarized compared to the P=O, owing to the lower electronegativity of sulfur compared to oxygen. Polarization of the P=O linkage (Fig. 9) results in a more electropositive phosphorus atom, which facilitates attack on phosphorus by nucleophilic agents, e.g., the serine hydroxyl of AChE. Organophosphorus esters containing the P=S moiety are less reactive and more stable to hydrolytic degradation than the corresponding P=O ester.

Investigations on the metabolism and mode of action of organophosphorus insecticides revealed that the toxicity of a P=S ester is attributed to the corresponding

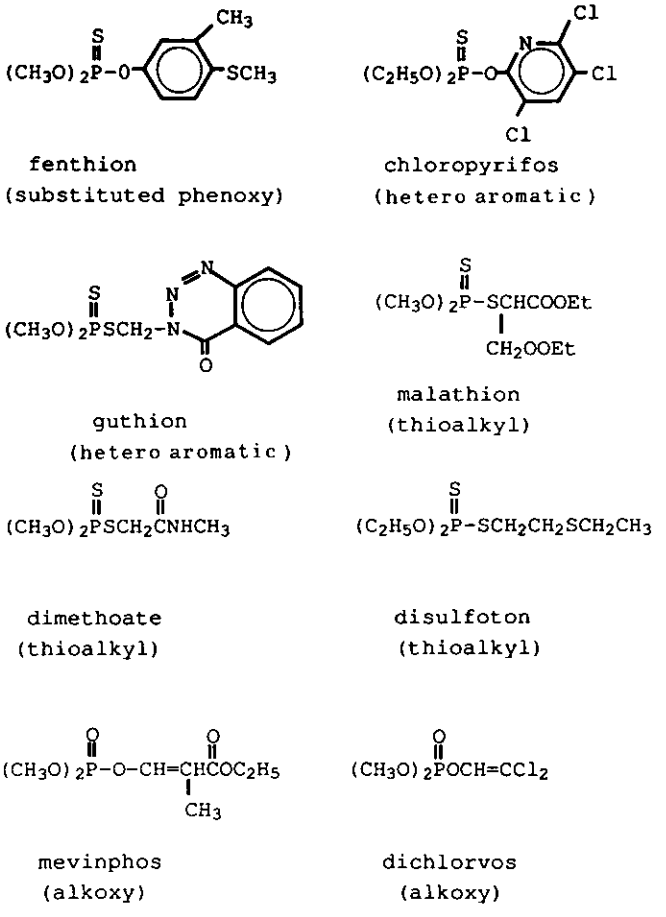


FIGURE 8. Different organophosphorus insecticides showing variation in the leaving group X.

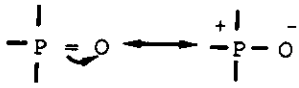
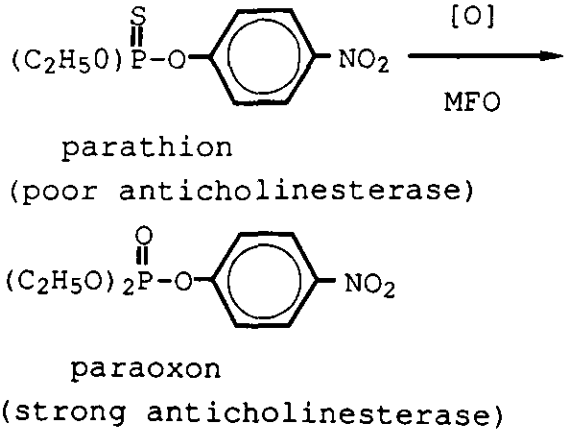


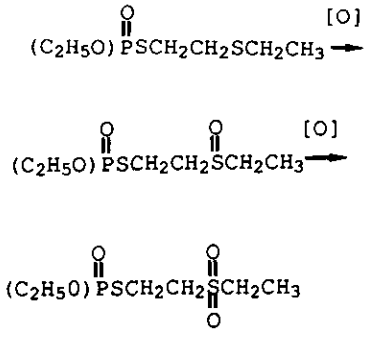
FIGURE 9. Polarization of the P=O linkage in organophosphorus esters.

P=O ester, formed by metabolic oxidation of P=S to P=O (*16,17*). This metabolic reaction is believed to be mediated by the mixed function oxidases (MFO), a ubiquitous enzyme system responsible for oxidation of foreign compounds in animals (*18*). A classical example of this activation reaction is found in the conversion of parathion (a poor anticholinesterase) to paraoxon (a strong anticholinesterase) [Eq. (4)].



(4)

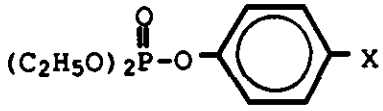
Another example of the effect of metabolic activation is evident in the toxicological data in Table 1 (*11*). The essentially identical housefly toxicity of these compounds in spite of the 10-fold differences in their bimolecular rate constant for inhibition (k_i) is readily explained on the basis of the metabolic activation of the thiomethyl moiety to the sulfoxide [—S(O)CH_3], which in turn is metabolized to the sulfone [—S(O)$_2CH_3$]. Thioether groups are highly susceptible to metabolic activation that proceeds through the sulfoxide and eventually to the sulfone. The metabolic oxidation of the thioether moiety in organophosphorus insecticides was first demonstrated in plants and animals with the demeton isomers, as indicated in Eq. (5) with the thiol isomer of demeton (*17,19*).



(5)

As in the case of the diethyl *p*-methylthiophenyl analogs in Table 1, demeton sulfoxide and sulfone were substantially stronger anticholinesterases than demeton, although all three compounds were equally effective insecticides. The addition of electronegative oxygen atoms to the thioether sulfur increases the electron withdrawing ability of this moiety, resulting in greater reactivity of the phosphorus atom.

Table 1. Toxicological data (*11*) for diethyl *p*-methylthio-, *p*-methylsulfinyl-, and *p*-methylsulfonyphenyl phosphates.



X	Fly-head AchE K_i/M/min	Housefly LD_{50}, μg/g
$-SCH_3$	1.4×10^3	2.5
$-S(O)CH_3$	1.5×10^4	1.5
$-S(O)_2CH_3$	1.5×10^5	2.0

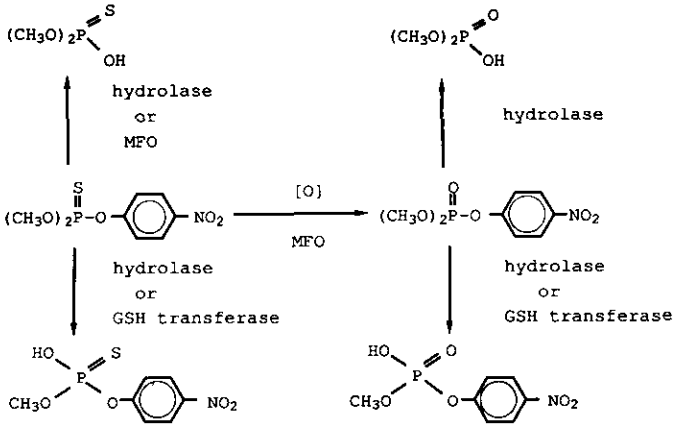


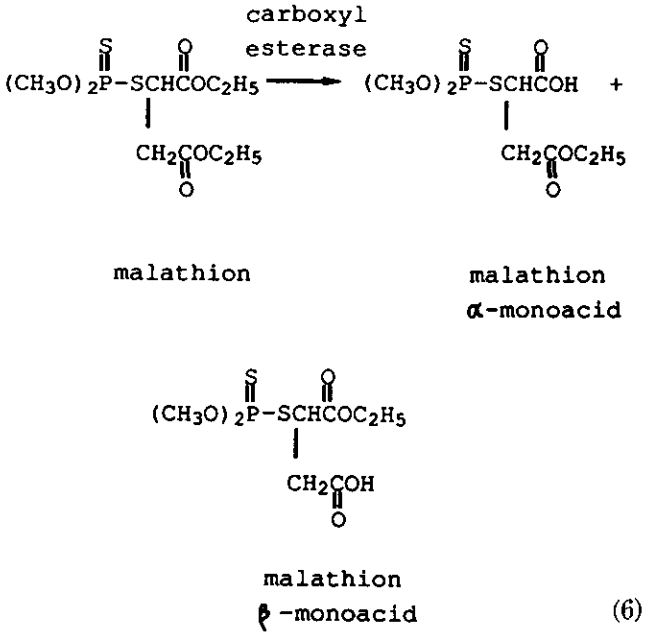
FIGURE 10. Reactions showing the metabolic degradation of methyl paraoxon and its activation product methyl paraoxon.

Metabolic Degradation

Anticholinesterase organophosphorus insecticides are, without exception, tertiary esters, and as tertiary esters they are susceptible to hydrolytic degradation, resulting in detoxication products. Detoxication of an organophosphorus insecticide may occur in a number of different ways. Cleavage of any bond attached to the phosphorus atom will lead to a detoxication product, e.g., by enzymatic or chemical hydrolysis (*20*). Enzymatic hydrolysis is mediated by a number of different esterases, which are generally referred to as hydrolases or phosphotriester hydrolases (*17*). Typical reactions for hydrolase catalyzed degradation of an organophosphorus insecticide are presented in Figure 10 in which methyl parathion is used as an example.

Metabolic degradation or detoxication may take place at a site away from the phosphorus center. The classical

example of this is found in the carboxylesterase catalyzed hydrolysis of malathion to its nontoxic carboxylic acid derivatives, as shown in Eq. (6). Detoxication to the monoacids occurs at a rate faster than that of the P=S to P=O activation reaction, and therefore malathion with a rat LD_{50} of about 3000 mg/kg is relatively safe to mammals. Malathion is toxic to insects owing to generally low concentrations of carboxylesterases in insects. Because of their susceptibility to hydrolytic degradation, organophosphorus insecticides are nonpersistent in the environment and in biological systems.

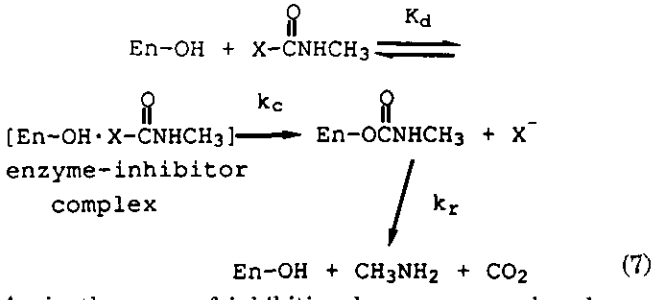


(6)

Carbamate Insecticides

Mechanism of Inhibition

The inhibition of AChE by a carbamate insecticide occurs by a mechanism virtually identical to that described earlier for an organophosphorus ester. The first step in the inhibition process involves the formation of the enzyme-inhibitor complex with subsequent carbamylation of the serine hydroxyl [Eq. (7)] resulting in inhibition of the enzyme (*21*).



(7)

As in the case of inhibition by an organophosphorus ester [Eq. (2)], the bimolecular inhibition constant k_i [not shown in Eq. (7)] is equal to k_c/K_d where k_c is the carbamylation rate constant (from complex to carbamylated enzyme), and K_d is the equilibrium constant for the complex dissociating back to reactants. In contrast to Eq. (2) for an organophosphorus ester, Eq. (7) contains a regeneration step (k_r) in which the carbamylated (inhibited) enzyme spontaneously regenerates to active enzyme, methylamine, and carbon dioxide.

Although the equations depicting the inhibition of AChE by a carbamate and organophosphorus ester are similar, there are distinct differences in the reaction between the enzyme and the two classes of compounds. First, while appropriate chemical reactivity is essential for high anticholinesterase activity for an organophosphorus ester, a good fit of the carbamate on the enzyme active site is essential for high anticholinesterase activity by a carbamate ester. This material will be discussed at greater length in the next section. Second, spontaneous regeneration of the carbamylated enzyme to active or original enzyme is relatively fast compared to spontaneous regeneration of a phosphorylated enzyme. For example, the half-life for recovery of *N*-methylcarbamylated AChE is approximately 30 min, while that for an organophosphorus ester ranges from several hours to days, depending upon the nature of the groups attached to the phosphorus atom [R and R′ in Eq. (2)]. In some cases AChE that is inhibited by certain types of organophosphorus esters is irreversibly phosphorylated and spontaneous regeneration does not occur.

Reactivity and Steric Properties

Most carbamate insecticides are derivatives of methylcarbamic acid and, therefore, are referred to as methylcarbamates. Insecticidal methylcarbamates are esters of substituted phenols and oximes (Fig. 11). Substituted phenols and oximes, each with pK_a values in the region of 10, give methylcarbamates that are reactive to the degree that they are able to carbamylate the serine hydroxyl of AChE. Owing to the intrinsic reactivity of methylcarbamate esters, an electron-withdrawing substituent is not required in the aryl moiety for high anticholinesterase activity. In fact, the introduction of a nitro substituent into the phenyl ring of phenyl methylcarbamate results in a compound of such high reactivity that it is hydrolytically degraded before it has an opportunity to inhibit the enzyme (*22*). It should be pointed out that methylcarbamate esters of alcohols (pK_a approximately 16) are intrinsically unreactive and, therefore, they are generally poor anticholinesterases.

A good fit of a methylcarbamate ester in the enzyme active site, i.e., formation of the enzyme-inhibitor complex prior to carbamylation, is crucial for high anti-

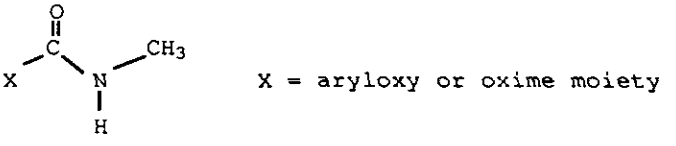


FIGURE 11. General structure of insecticidal methylcarbamates.

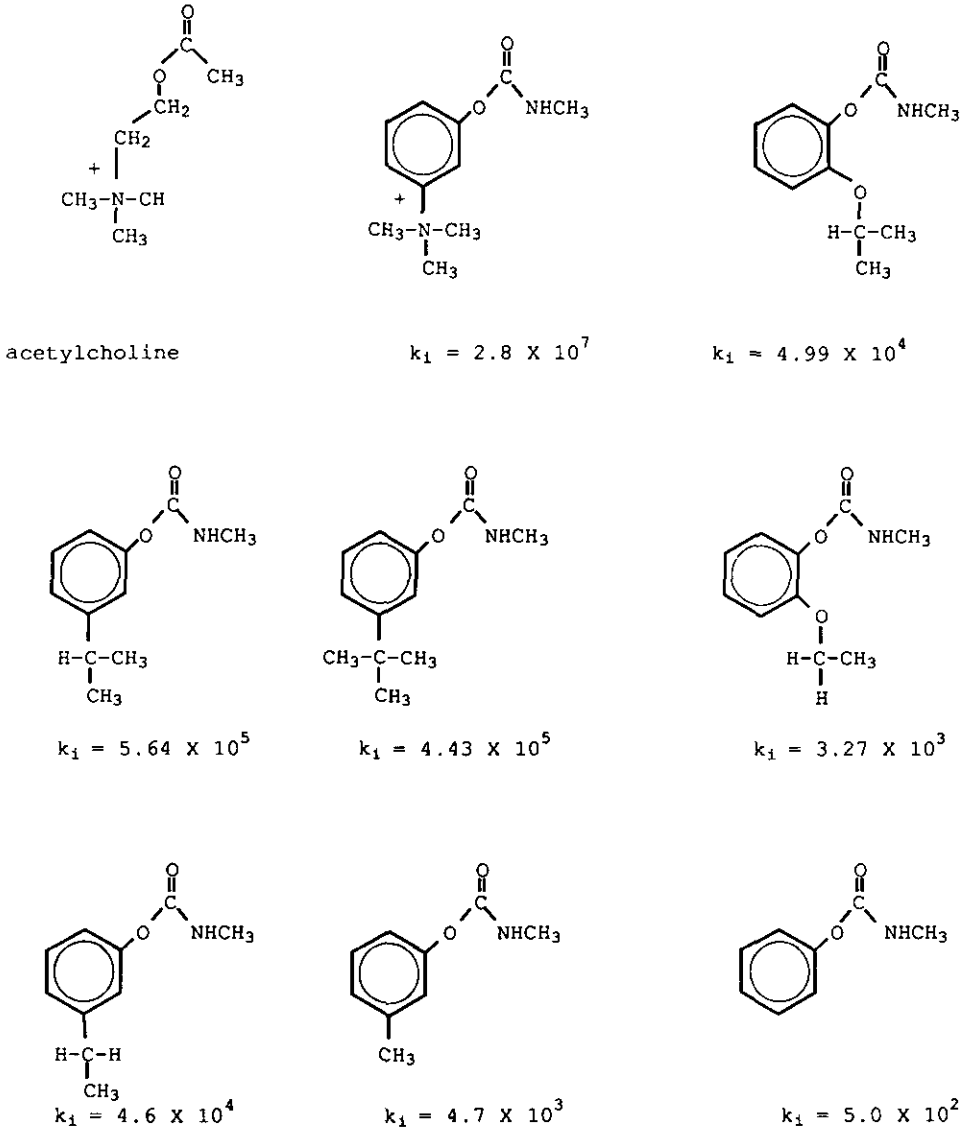


FIGURE 12. Structure and anticholinesterase activities of different substituted phenyl methylcarbamates. k_i values/M/min are for bovine erythrocyte AChE. Data from Nishioka et al. (*22*) and Reiner and Aldridge (*23*).

Table 2. Dissociation (K_d) and rate constants (k_i and k_c) for the *inhibition of bovine erythrocyte AChE by substituted phenyl* methylcarbamates.[a]

Ring substituents	K_d,M	k_c, min^{-1}	k_i, $M^{-1}min^{-1}$
3-Trimethylammonium	—	—	2.8×10^7
2-Isopropoxy	3.97×10^{-5}	1.98	4.99×10^4
2-Ethoxy	1.62×10^{-4}	0.53	3.27×10^3
3-*tert*-Butyl	3.20×10^{-6}	1.42	4.43×10^5
3-Isopropyl	5.55×10^{-6}	3.13	5.64×10^5
3-Ethyl	6.45×10^{-4}	2.22	3.43×10^4
3-Methyl	3.76×10^{-3}	1.40	3.72×10^3
Unsubstituted	3.02×10^{-3}	0.86	2.84×10^2

[a] The K_d, k_c, and k_i values for all ring substitutes except 3-trimethylammonium are from Nishioka et al. (*22*) and the k_i value for 3-trimethylammonium from Reiner and Aldridge (*23*).

cholinesterase activity. Evidence in support of this is found in the values of K_d, k_c and k_i determined for a wide spectrum of substituted phenyl methylcarbamates with representative values given in Table 2 (*22,23*). According to the data, the overall bimolecular inhibition constant k_i for these methylcarbamates is almost totally dependent on K_d, the equilibrium constant for dissociation of the enzyme-inhibitor complex. K_d and k_c data were not available for the 3-trimethylammonium analog. K_d may be regarded as an affinity constant, i.e., the smaller the value of K_d, the tighter the complex. Carbamate esters with small values for K_d were strong anticholinesterases, and those with large K_d were poor anticholinesterases. In contrast to K_d, which was highly variable in value, k_c, the rate constant for the carbamylation step [Eq. (7)], showed relatively little variation, indicating similar levels of reactivity for the different methylcarbamates.

Methylcarbamates of substituted phenols and oximes with good complementary fit to the enzyme active site are generally strong inhibitors of AChE (*21*). Com-

pounds that structurally resemble acetylcholine, the natural substrate for AChE, invariably are strong inhibitors. This is made apparent by the structures of the substituted phenyl methylcarbamates in Table 2 and their anticholinesterase activities as presented in Figure 12. The structure of acetylcholine is included to show spatial similarities between it and carbamates with strong anticholinesterase activities. Noteworthy is the approximately 10-fold increase in anticholinesterase activity as the three-substituent is increased in size from hydrogen, methyl, ethyl to isopropyl ≥ *t*-butyl. Evidently maximum hydrophobic interaction of the ring substituent with the enzyme active site is reached when two methyl groups are attached to the central carbon atom. Moreover, replacement of the central carbon atom with a positively charged quaternary nitrogen atom resulted in about a 50-fold increase in anticholinesterase activity (compare *1* with *4* in Table 2), attributable to electrostatic attraction between the positive nitrogen atom and the negative charge in the anionic site. It should be added that results similar to those given in Table 2 were also observed for the inhibition of insect AChE (*24*).

Oxime methylcarbamates, represented by aldicarb and methomyl (Fig. 13), are structurally similar to acetylcholine, good inhibitors of AChE, and also potent insecticides. Another important methylcarbamate insecticide, carbofuran, is structurally closely related to 2-isopropoxyphenyl methylcarbamate (propoxur, compound *2* in Fig. 12) but where the isopropoxy moiety is bridged to the ring by a methylene. In this case the *gem*-dimethyl group is rigidly fixed at an optimum distance from the carbamate moiety, allowing maximum interaction with the enzyme active site. Carbofuran is about 10-fold more potent in inhibiting AChE than propoxur and is one of the most effective carbamate insecticides.

Activation and Degradation

The principal route of metabolism of carbamate insecticides in animals is oxidative in nature and is generally associated with the MFO enzymes. Typical oxygenation reactions in which an oxygen atom is introduced into the molecule include hydroxylation of aromatic rings, *O*-dealkylation of ethers, *N*-methyl hydroxylation and demethylation, oxidation of aliphatic side chains, and thioether oxidation (*25,26*). However, the metabolism of aldicarb to its sulfoxide is believed to be an activation reaction, since aldicarb sulfoxide is approximately 10-fold more potent as an anticholinesterase than aldicarb. Methylcarbamate insecticides are

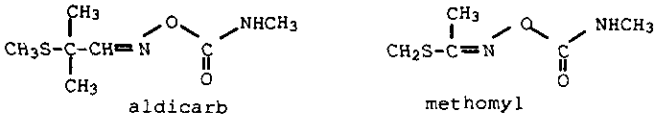


FIGURE 13. Structure of the oxime methylcarbamates aldicarb and methomyl.

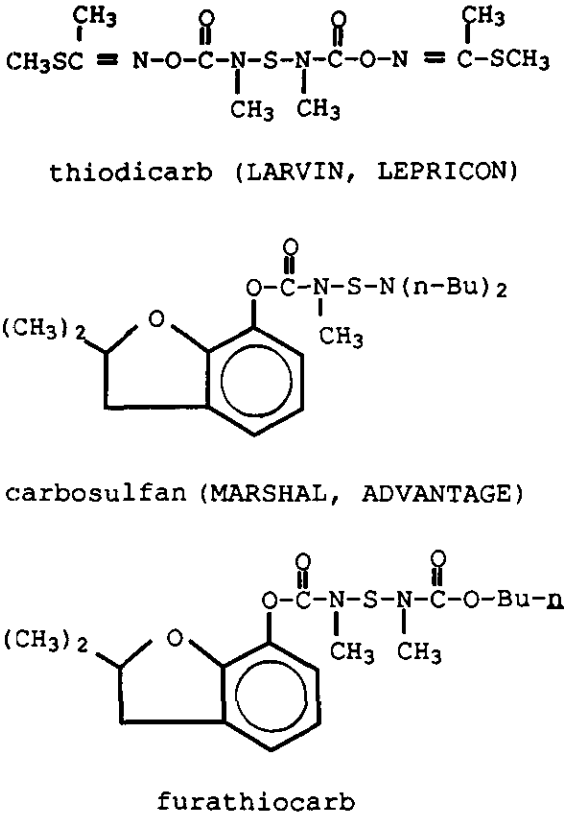
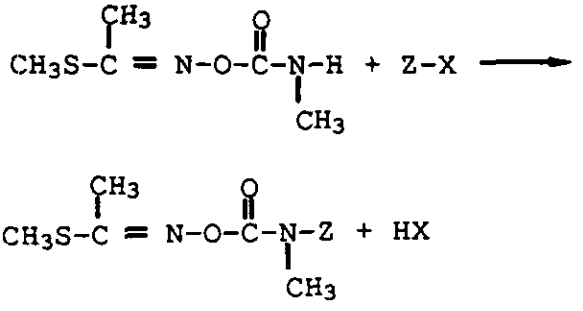


FIGURE 14. Structures of procarbamate insecticides.

also highly susceptible to alkaline hydrolysis but are relatively stable to neutral or acidic conditions (*27*).

Methylcarbamate insecticides are, on the whole, relatively toxic to mammals. One of the reasons for their high acute toxicity is that they are direct inhibitors of AChE, and metabolic activation is not required as in the case of many safe organophosphorus insecticides, e.g. malathion. In order to improve the toxicological properties of methylcarbamate insecticides, a number of these compounds have been converted to derivatives by replacement of the hydrogen atom on the carbamate nitrogen with an appropriate functional group (*28*). The reaction leading to derivatized methylcarbamates is illustrated by Eq. (8) using methomyl as the starting carbamate.



(8)

Z may be represented by a wide variety of nucleophiles, many having a nucleophilic sulfur atom. The replace-

ment of hydrogen with Z usually results in dramatic reduction in anticholinesterase activity along with substantial improvement in acute mammalian toxicity, which is attributed to the delayed factor provided by Z that allows alternative routes for detoxication in mammals. In insects, the N—Z bond is rapidly broken, leading to *in vivo* generation of the parent methylcarbamate and intoxication of the insect. These derivatives are referred to as procarbamate insecticides and are represented by structures given in Figure 14.

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