Biodegradation and Detoxification of Environmental Pollutants

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CRC Press, Inc.
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Library of Congress Cataloging in Publication Data

Main entry under title:

Biodegradation and detoxification of environmental pollutants.

Includes index.
1. Microbial metabolism. 2. Xenobiotic metabolism. 3. Pollution. 4. Chemicals—Metabolic detoxication. 5. Biodegradation.
I. Chakrabarty, Ananda M., 1938- .
II. Title: Detoxification of environmental pollutants.
QR88.B56 576'.1133 81-15505
ISBN 0-8493-5524-9 AACR2

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

1982 by CRC Press, Inc.

International Standard Book Number 0-8493-5524-9

Library of Congress Card Number 81-15505
Printed in the United States

PREFACE

The basic objective of this book is to define and review the recent advances in our understanding on the biological mechanisms in the biodegradation and detoxification of various environmental pollutants. Thus, the enzymes involved in the hydroxylation and detoxification of many carcinogenic polycyclic aromatic hydrocarbons by various mammalian tissues have been widely studied, and the genetic basis of such hydroxylation has been delineated. The toxicological problems created by such persistent halogenated hydrocarbons as Kepone® or PBBs have focused attention on the desirability of a detailed study of the biodegradability of such compounds in the environment. Considerable progress in the understanding of the enzymatic breakdown of various chlorinated molecules and pesticides both by aerobic and anaerobic microorganisms, has been made in recent years. Extra chromosomal genetic elements are now known to be involved in the biodegradation of various chlorinated and nonchlorinated hydrocarbons. The involvement of repeated sequences, as well as transposability of plasmid genes, in the evolution of microbial biodegradative functions are also discussed. Finally, various toxic organometallic compounds are released into the environment in the form of fungicides or as by-products of industrial operation. Detailed enzymatic and genetic mechanisms involving reduction or methylation of mercury and its derivatives have been elucidated in recent years. The book encompasses recent studies on the mode of detoxification of such mercurial compounds by various microorganisms.

THE EDITOR

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Chapter 1

MICROBIAL METABOLISM AND ENZYMOLOGY OF SELECTED PESTICIDES

D. M. Munnecke, L. M. Johnson, H. W. Talbot, and S. Barik

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I. INTRODUCTION

The use of synthetic organic chemicals in agricultural practices increased dramatically in the years immediately following World War II. The need to know the fate and effect of these new chemicals in the agricultural environment created tremendous research efforts. Early investigators were primarily concerned with determining which degradative processes were important in establishing the stability of these molecules in the environment. When it was realized that microorganisms played an important role in the degradation of these xenobiotics, more directed biochemical and microbiological projects were initiated to determine the basic principles of microbial metabolism. Thus throughout the 1950s and 1960s, investigators were involved with associating microbial metabolic activities with pesticide transformation. This led some scientists to be amazed at the ability of microbes for degrading these newly synthesized, unique chemicals, claiming that microbes were infallible and could degrade any synthetic molecule, while others were more skeptical and believed that certain molecules could not be metabolized and were therefore considered recalcitrant.[1,2] This debate was tremendously invigorating to the field of pesticide metabolism, since it led investigators toward a better understanding of the basic principles concerning the enzymology and biochemistry of pesticide-related metabolism. This argument also helped to establish the fact that the persistence and fate of these xenobiotics in the environment were strongly affected by microbial activity, and in many situations, microbes were more instrumental in pesticide degradation than were physical or chemical processes.

In the 1970s the mood of the pesticide industry changed from an earlier optimistic feeling to one of recognition of the damages that tremendous large-scale use of these agricultural chemicals could do to our environment as well as to public health. Pesticide usage had increased from 1.1×10^9 lb/year in 1971 to 1.4×10^9 lb in 1977, and to 1.5×10^9 lb by the end of the decade.[3] Problems of chronic exposure,[4] of improper waste disposal,[5] environmental damage,[6] and many more adverse responses to pesticides required that federal laws be promulgated to govern the manufacture, distribution, and use of agricultural chemicals. These laws were aimed at restricting the use of extremely toxic or environmentally dangerous chemicals and reducing the undesirable discharge of pesticides into the environment. In developing these laws, the metabolism studies from the 1950s to 1960s were very helpful in determining which agricultural chemicals, due to their environmental stability and inherent toxicity, truly represented a danger to our chemically dependent society. The new federal laws enacted to control these hazardous chemicals required better technology for control of pesticide waste discharges and for pesticide cleanup. The tremendous diversity in the type of chemicals to be detoxified mandated that many different approaches be considered. Physical methods of incineration, entrapment, and burial were developed,[7,8] as well as chemical methods involving oxidation, reduction, and hydrolysis.[9] However, heavy use of biological treatment systems persisted, and this technology was improved. An important aspect of earlier pesticide metabolism studies helped to develop a new approach to biological pesticide waste treatment technology. Crude enzyme extracts from microorganisms were, in many investigations, able to hydrolyze pesticides to less toxic and less persistent compounds. And the expanding field of biotechnology helped to strengthen the idea that microbial enzymes could be used to control the fate of pesticides in the environment. Using the knowledge gained from research in the 1960s, efforts in the late 1970s led to the demonstration of the potential use of an organophosphate-hydrolyzing enzyme for a variety of detoxification applications, including pesticide detoxification in industrial wastewaters, pesticide containers, and in soils after accidental spills.[10,11]

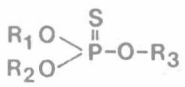
The intent of this chapter is to review the scientific literature pertaining to the me-

Table 1
TOXICITY OF SELECTED PESTICIDES AND THEIR MAJOR METABOLITES

Pesticide	Major metabolite	Toxicity (mg/kg)[a]	Detoxification factor
Organophosphates			
Paraoxon	—	1.8	
	p-Nitrophenol	350	386
Parathion	—	6	
	p-Nitrophenol	350	122
Dithioates			
Azinphos-methyl	—	13	
	Anthranilic acid	4620	824
Carbamates			
Carbaryl	—	500	
	1-Naphthol	2590	7
Phenylureas			
Linuron	—	1500	
	3,4-Dichloroaniline	100	−23
Monuron	—	1480	
	4-Chloroaniline	300	−8
Acylanilides			
Alachlor	—	1800	
	2,6-Diethylaniline	2690	2
Propanil	—	560	
	3,4-Dichloroaniline	100	−8
Phenoxyacetates			
2,4-D	—	370	
	2,4-Dichlorophenol	580	2
Silvex	—	650	
	2,4,5-Trichlorophenol	320	2

[a] Toxicity reported: LD_{50} oral, rat, mg/kg, data from Christensen.[12] Dash denotes increase in toxicity.

tabolism of various pesticides by cell-free enzyme extracts in order to show the potential that microbial enzyme systems have in regard to the detoxification and disposal of pesticides in industrial situations. Our basic postulation is that microbial enzymes can be isolated and be effective in converting toxic or persistent pesticides into less toxic or less persistent molecules. For pesticides belonging to the organophosphates, dithioates, carbamates, acylanilides, phenylureas, and phenoxyacetates, hydrolases are instrumental in initial steps of whole cell metabolism. Hydrolases are prime candidates for industrial applications because they are generally stable enzymes and by definition, do not require co-factors. In Table 1, the toxicity of some selected pesticides is listed, as well as the toxicity of their major enzymatic hydrolysis product.[12] For instance, the hydrolysis of parathion to *p*-nitrophenol leads to a 122-fold decrease in overall toxicity and complete removal of parathion. For organophosphates, dithioates, and carbamates, a hydrolysis of the parent pesticide will generally lead to a significant reduction in toxicity. For the other three classes of pesticides shown in Table 1 and discussed in this chapter, the acylanilides, phenylureas, and phenoxyacetates, hydrolysis does not generally lead to a significant reduction in toxicity and sometimes leads to more toxic chemicals. Yet the biospecificity of the pesticide molecule is destroyed, and the metabolites are not as stable in the environment as the pesticide molecule. After the reader has been acquainted with the enzymology of pesticide metabolism, the potential industrial applications of this enzyme technology can be discussed and conclusions be made in regard to the future development of this field of biotechnology.



where R_1 and R_2 = alkyl (either ethyl or methyl) moiety

R_3 = substituted aryl or alkyl moieties

FIGURE 1. General chemical structure of organophosphate pesticides.

II. ORGANOPHOSPHATES

A. Metabolism

Pesticides belonging to the organophosphates have recently been extensively used as replacement chemicals for the more persistent organochlorine and mercurial compounds. Excellent reviews have been published discussing the environmental fate of organophosphate pesticides and the role microorganisms play in their degradation.[13-18] The organophosphates are characterized as having a general formula of the type shown in Figure 1. In this class, parathion (O,O-diethyl-O-*p*-nitrophenyl phosphorothioate) is the most extensively studied insecticide due to its intensive agricultural use. Indirect evidence of microbial participation in the degradation of parathion was shown by examining the stability of parathion in autoclaved and nonautoclaved soil and water samples.[19,20] In some cases, heat-labile agents of microbial origin were isolated from the soil, which helped in rapid degradation of parathion. Methyl parathion, like ethyl parathion, also disappeared rapidly in nonsterilized soils but not in sterilized soil.[21] Conclusive evidence for the involvement of microorganisms in parathion degradation was obtained when microbes were isolated from soil, and subsequently in vitro metabolism studies were performed.

Parathion degradation can proceed through basically three different pathways, depending on environmental conditions and the microorganisms involved. Parathion can undergo a nitro group reduction forming amino parathion[18] or an ester hydrolysis forming *p*-nitrophenol and diethylthiophosphoric acid (Figure 2).[18,22] Formation of amino parathion depends upon the organic matter content of the soil, the oxidation-reduction potential, and more specifically, upon the interaction with certain microflora-like yeasts and fungi.[18] The metabolism of parathion to amino parathion creates a more persistent compound.[23,24] Hydrolysis of the phosphoester bond, however, can lead to complete mineralization of the parent molecule. Before hydrolysis, parathion can be oxidized to paraoxon, which can subsequently undergo hydrolysis to *p*-nitrophenol and diethylthiophosphoric acid (Figure 2).

In order to examine microbial metabolism of parathion, various isolation and enrichment techniques were used to obtain microbes capable of metabolizing this compound. Enrichment of microbial populations after repeated applications of parathion to an alluvial soil was demonstrated with the isolation of a bacterial culture (*Pseudomonas* sp. ATCC 29353) capable of using parathion as a sole source of carbon and energy.[25,26] By slight modification of enrichment techniques, bacterial cultures were isolated that could metabolize parathion to diethylthiophosphoric acid and *p*-nitrophenol, or to amino parathion.[27,28] *p*-Nitrophenol was subsequently metabolized by some of these cultures.

Hsieh and Munnecke[29] used a chemostat to isolate microbial cultures capable of detoxifying parathion. A continuous fermentation process was used, and a mixed bacterial culture was adapted to parathion metabolism. None of the bacterial isolates uti-

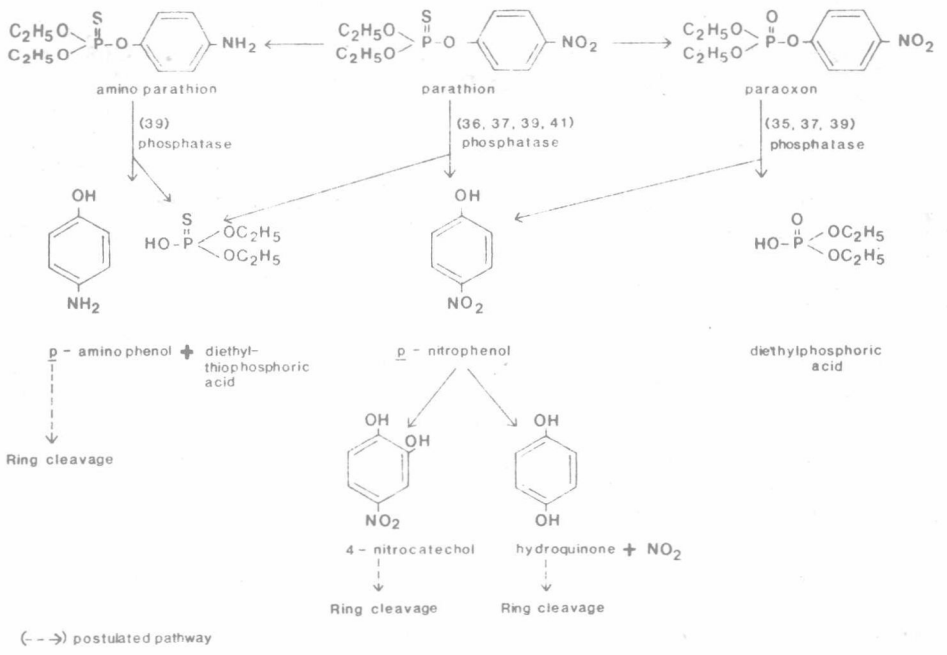


FIGURE 2. Pathways for the metabolism of parathion.

lized parathion as a sole carbon source although eight of the nine cultures utilized *p*-nitrophenol as the sole source of carbon and energy.[30] In a subsequent study, Daughton and Hsieh[31] isolated two *Pseudomonas* species from the mixed culture, *P. stutzeri* which cometabolically hydrolyzed parathion to *p*-nitrophenol, and *P. aeruginosa*, which utilized this nitrophenol as a sole carbon source. Methyl parathion was also metabolized in an analogous manner.

B. Enzymology

In general, the initial point of organophosphate metabolism is the hydrolysis of the arylphosphoester bond. The aromatic metabolites can subsequently be oxidized to produce energy and substrates for cellular growth. Thus this hydrolase (EC 3.1.3), commonly referred to as an esterase, arylesterase, or phosphotriesterase, is the most important enzyme in bacterial metabolism of organophosphates. An analogous enzyme has been extensively studied in both insects and mammals[32] since this reaction is a major mechanism of resistance and detoxification. However, only microbial studies will be reviewed in this chapter. In one of the earliest studies of organophosphate hydrolases, Mounter et al.[33] reported that a group of microorganisms contained enzymes capable of hydrolyzing dialkylfluorophosphates. The DFPase activity (EC 3.7.2.1.) (see Table 2) was found in 10 of 14 tested microbes and was activated by Mn^{2+} and inhibited by Co^{2+}.

The DFPase obtained from *Escherichia coli, Pseudomonas fluorescens, Streptococcus faecalis,* and *Propionibacterium pentosaceum* hydrolyzed fluorophosphate, pyrophosphate, and dimethyl chlorovinyl phosphate compounds at appreciable rates, but failed to hydrolyze dialkylaryl phosphates (trichlorfon, paraoxon), dialkylaryl thiophosphates (parathion, chlorthion) and triethyl phosphates (Table 2).[34]

In another study, Zech and Wigand[35] isolated two phosphohydrolases from *E. coli.* One of the enzymes, DFPase (EC 3.8.2.1) could detoxify DFP (see Appendix) and the

Table 2
ENZYMATIC ACTIVITY OF MICROBIAL CELL-FREE EXTRACTS FOR PESTICIDE DEGRADATION

Pesticide	Enzyme class	Enzyme activity (nmol of substrate transformed/min/ mg protein)	Ref.
Organophosphates			
Acephate	Esterase	52	41
Aspon	Esterase	110	41
Cyanophos	Esterase	58	39
Diazinon	Esterase	301, 1200, a	36, 39, 41
Dursban	Esterase	600	39
DCVMP	Lyase	a	34
DEFP	Lyase	a	34
DFP	Lyase	a	34
EPN	Esterase	12	39
Fenitrothion	Esterase	217	39
Fensulfothion	Esterase	238	41
Methyl parathion	Esterase	600, a	37, 39
Monocrotophos	Esterase	133	41
Paraoxon	Esterase	13, 3600, a	35, 37, 39
Parathion	Esterase	259, 3000, 7000, a	36, 37, 39, 41
Propetamphos	Esterase	50	40
Quinalphos	Esterase	1410	40
Triazophos	Esterase	4350	39
TEPP	Lyase	a	34
Dithioates			
Azinphos-methyl	Esterase	87	58
Carbophenothion	Esterase	142	41
Dimethoate	Esterase	62	58
Malathion	Esterase	13, 665, 288, a	41, 56, 57, 58
Malathion	Phosphatase	a	49
Carbamates			
Chlorpropham	Esterase	6	83
Propham	Acylamidase	8	92
Propham	Esterase	5	83
PPG-124	Acylamidase	67	106
Phenylureas			
Carboxin	Acylamidase	252	91
Chlorbromuron	Acylamidase	11, 15	91, 92
Linuron	Acylamidase	18, 20, 130	88, 91, 92
Metabromuron	Acylamidase	16, 18	88, 91
Monalide	Acylamidase	29, 238	91, 92
Monolinuron	Acylamidase	15, 20	91, 92
Monuron	Acylamidase	4	88
Pyracarbolid	Acylamidase	35	91
Acylanilides			
Dicryl	Acylamidase	0.2	96
Karsil	Acylamidase	0.4	96
Propanil	Acylamidase	2, 3, 555	92, 96, 106
Solan	Acylamidase	0.3	96
Phenoxyacetates			
2,4-D	Oxygenase or hydrolase	0.1	114
MCPA	Oxygenase or hydrolase	0.1	127

Note: a = insufficient data to calculate enzyme activity.

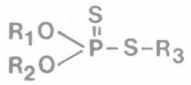
other, paraoxonase (EC 3.1.1.2) hydrolyzed paraoxon (Table 2). DFPase could not hydrolyze paraoxon and vice versa. Both enzymes had a very low substrate specificity ($K_m = 1.7 \times 10^{-2}$ and 5×10^{-3} m/ℓ for DFP and paraoxon, respectively), had activity at low pH (8 to 8.5), and were unstable at room temperature.

A cell-free preparation from *Flavobacterium* sp. ATCC 27551 described by Sethunathan and Yoshida hydrolyzed diazinon, parathion, and chlorpyrifos.[36] In all three cases, the aromatic or heterocyclic moiety produced by hydrolysis could not be further metabolized by this bacterium. This phosphoesterase (EC 3.1.3) did not hydrolyze amino parathion or malathion, a dithioate insecticide. In a recent investigation with this *Flavobacterium* sp., Brown[37] reported that the constitutive phosphotriesterase was composed of two protein units, one with a molecular weight greater than 100,000 daltons and the other with a molecular weight of 50,000 daltons. The larger molecule was associated with an orange gum which probably contained flavoprotein. The enzyme was active with those organophosphates having an electron withdrawing aromatic or heterocyclic leaving group as in parathion, paraoxon, and diazinon. The enzyme could not hydrolyze compounds with weakly electrophilic groups like 4-aminophenyl. This phosphoesterase showed activity over a broad range of pH values (8 to 10) and was unaffected by the presence of EDTA, NaF, or NaN_3 or metal ions such as Zn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Mg^{2+}, or Mn^{2+}. Irreversible enzyme inhibition was seen with non-ionic detergents (0.16%) such as Triton® X-100 and Tween® 80.

In a study by Adhya et al.,[38] this same *Flavobacterium* sp. hydrolyzed both methyl parathion and fenitrothion in addition to diazinon and parathion (Table 2). A *Pseudomonas* species ATCC 29353 isolated from flooded soil[26] was more specific in organophosphate hydrolysis and could hydrolyze parathion and diazinon but not methyl parathion and fenitrothion, two dimethyl aryl thiophosphates. Evidently, the phosphotriesterases from these two organisms are quite different in their specificity even though both hydrolyze the same phosphoester bond.

A crude enzyme extract obtained from a mixed bacterial culture that was grown on parathion showed exceptionally high activity for hydrolysis of at least ten other organophosphates (Table 2).[39,40] The rate of parathion hydrolysis, for example, was 3000 nmol/min/mg protein and was 3000 times faster than chemical hydrolysis by 0.1 *N* NaOH. This phosphoesterase (EC 3.1.3) was unaffected by high substrate concentration, or metabolite accumulation, did not require co-factors, and was active between pH 7.0 to 10.5 with optimal activity at pH 8.5. The temperature optimum of the enzyme was 35°C. This crude enzyme preparation was stable at room temperature in aqueous solution but was denatured by freezing. The presence of various solvents at 15 to 450 ppm accelerated hydrolysis, but at 1000 ppm solvent concentrations the enzymatic activity was reduced. Cleavage of the aryl phosphoester bond was influenced more by alkyl substituents on the phosphorous atom than by functional groups on the aromatic ring. In the case of diazinon, EPN, chlorpyrifos, and triazophos, the effect of aromatic functional groups did not prevent enzymatic hydrolysis, but a $-SCH_3$ group in the para position (fenthion) inhibited hydrolysis. Both dimethyl- and diethyl-substituted dithiophosphates (ethion, azinphos-methyl, chlormephos) were not hydrolyzed by this crude enzyme preparation.

Munnecke[11] covalently bound this crude enzyme extract to porous glass and silica beads and examined the properties of the immobilized enzyme for the detoxification of industrial wastewaters and concentrated pesticide solutions. The immobilized enzyme exhibited approximately 50% of its soluble enzyme activity and displayed minor changes from the characteristics of free enzyme in regard to differences in pH and temperature. An immobilized enzyme reactor could hydrolyze 90% of a 10 ppm parathion solution at flow rates up to 96 ℓ/hr.



where R_1 and R_2 = alkyl (either ethyl or methyl) moiety
R_3 = substituted alkyl or aryl moieties

FIGURE 3. General chemical structure of dithioate pesticides.

In another study, Rosenberg and Alexander[41] obtained an inducible phosphoesterase from two *Pseudomonas* species grown in the presence of either diazinon or malathion as sole phosphorous source. A crude enzyme preparation could hydrolyze aspon, monocrotophos, fensulfothion, diazinon, malathion, acephate, parathion, and trithion, but could not hydrolyze dimethoate, trichlorfon, methyl parathion, or dichlorvos (Table 2) (see Appendix). The cleavage of the common phosphorous-oxygen bond of these pesticides was not influenced by the type of alkyl substituents since methyl, ethyl, and propyl esters were metabolized. This enzyme was active against the dithioate insecticides malathion and carbophenothion in contrast to the activity of the parathion hydrolase reported earlier by Munnecke.[39]

All of the above organophosphate hydrolysis studies dealt with the primary hydrolysis of the parent molecule. Clearly this is an important step toward the partial or complete mineralization of these pesticides. For example, *p*-nitrophenol, the major metabolite formed by hydrolysis of parathion, paraoxon, or methyl parathion is readily metabolized by numerous microorganisms, as are other phenol metabolites from other organophosphates.[30,42-45] Cleavage of the aromatic ring after ring hydroxylation involves oxygenases and leads to the formation of intermediates which eventually enter the Krebs cycle. These aromatic pathways have been reviewed elsewhere.[46]

III. DITHIOATES

A. Metabolism

Dithioates constitute a major group of organophosphates. They are characterized by the general chemical formula shown in Figure 3. The pesticides belonging to this group are in great demand due to their broad spectrum and high insecticidal activities. However, not much work has been reported regarding their microbial metabolism except perhaps for one compound, malathion [O,O-dimethyl-S-(1,2-dicarbethoxyethyl)phosphorodithioate].

The hydrolysis of malathion can be both chemical and biological in nature.[47,48] Chemical hydrolysis, as expected, is pH and temperature dependent,[14] but most studies indicate that the major pathway of malathion disappearance in soil, water, sediments, and salt marsh environments is biologically mediated.[47,49-52]

Bacterial cultures isolated from malathion-acclimated estuarine environments degraded malathion either to malathion half-ester or to malathion dicarboxylic acid through half-ester intermediates (Figure 4). One bacterium isolated from a parathion enrichment culture degraded malathion to malathion diacid via half-ester intermediates.[52] The formation of carboxylic acids strongly suggests the activity of carboxyesterase in malathion metabolism.

Malathion disappeared rapidly from salt marsh environments due to bacterial involvement.[49,50] Several bacterial cultures isolated from this ecosystem utilized malathion with or without additional nutrients and formed malathion mono- and dicarboxylic acid, which indicated carboxyesterase enzyme activity. In another experiment, a

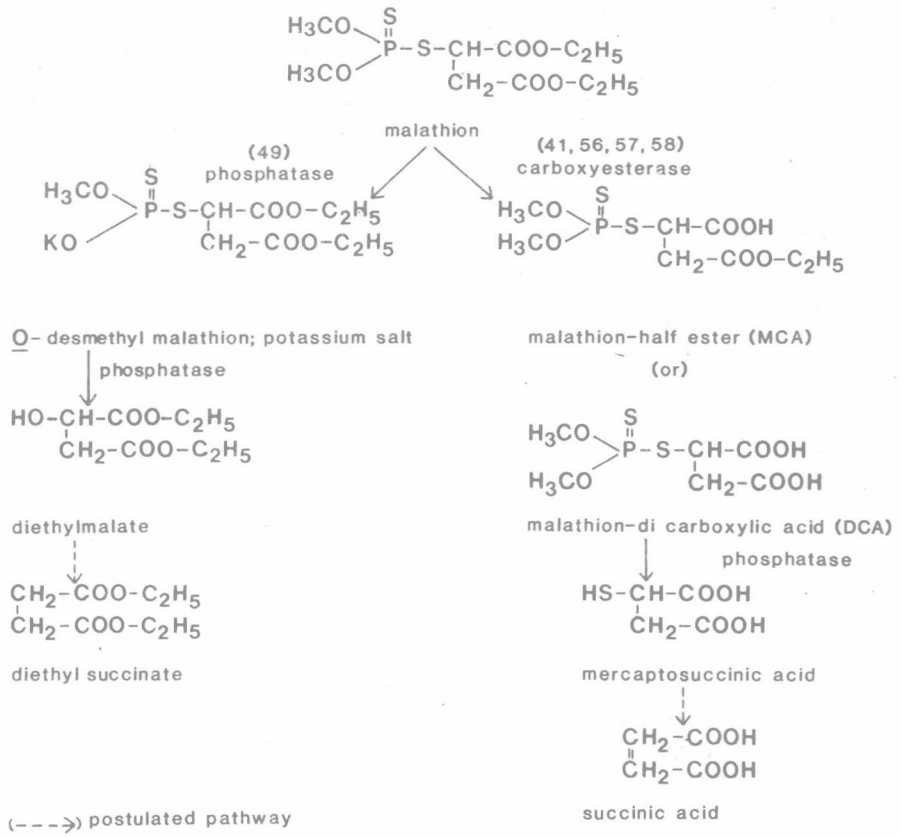


FIGURE 4. Proposed pathway for the metabolism of malathion.

mixed bacterial population utilized malathion as a sole carbon source and formed B-monoacid as the major metabolite.[53] The fungus *Aspergillus oryzae* was isolated from a freshwater pond and also produced B-monoacid and malathion dicarboxylic acid.[54] Both bacterial and fungal cultures had carboxyesterase activity which hydrolyzed the parent molecule to carboxylic acids. These intermediary products were subsequently metabolized to inorganic phosphate and thiophosphates (Figure 4). Demethylation of malathion in bacterial[49,50] and in fungal systems[51,55,56] has been reported. This reaction occurred in some instances after the carboxyesterase reaction, and in one instance demethylation was reported to be a chemical reaction.[48]

B. Enzymology

Two species of the genus *Rhizobium*, *R. leguminosarum* and *R. trifolii*, were isolated from an Egyptian soil and had powerful carboxyesterase activity (EC 3.1.1.1) toward malathion.[55] Five hydrolytic metabolites, inorganic phosphate and thiophosphate, were identified as metabolites. In a study by Mostafa et al.[51] involving three fungal isolates — *Aspergillus niger*, *Penicillium notatum* and *Rhizoctonia solani* — the presence of carboxyesterase activity was indicated when malathion was degraded to different carboxylic products which constituted nearly 40% of the total metabolites. Monomethyl phosphate and thiophosphate formed in the culture medium due to the action of two other enzymes, an esterase (EC 3.1.1.2) and a phosphatase (EC 3.1.3). The esterase cleaved the sulfur carbon bond of the mono- and di-acids to dimethyl

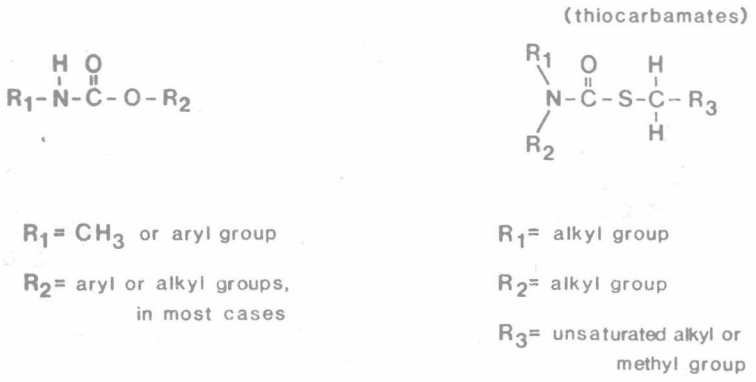


FIGURE 5. General chemical structure of carbamate pesticides, including thiocarbamates.

phosphorothioates and dimethyl phosphorodithioates, which ultimately demethylated through phosphatase activity to these products. This clearly suggests the establishment of these enzymes in cultures metabolizing malathion.[51]

Matsumura and Boush[56] examined a strain of *Trichoderma viride* and a *Pseudomonas* sp. and found that malathion was rapidly metabolized by the action of a soluble carboxyesterase (EC 3.1.1.1), as evidenced by the presence of carboxylic acid derivatives in the culture medium in addition to other demethylated and hydrolytic products.

Cell-free enzymatic hydrolysis of malathion at the phospho-sulfur bond was reported by Rosenberg and Alexander (Table 2).[41] This enzyme, obtained from two *Pseudomonas* species grown on either diazinon or malathion, hydrolyzed carbophenothion and malathion, but failed to metabolize dimethoate. An alkali-extractable, heat-labile organic entity from soil, which transformed malathion to malathion monoacid, was isolated by Getzin and Rosefield[57] from both irradiated and nonirradiated soil. This soil-free, extracellular esterase was quite stable, had a pH optimum of 6.8, and displayed normal Michaelis-Menten kinetics. The enzyme was not denatured with increased acidity (<2.0) and was active over a wide temperature range (20 to 70°C).

A program has been initiated in our laboratory to obtain bacterial cell-free enzymes with specificity for dithioate pesticides. Several bacterial and fungal isolates have been obtained from soil and sewage through enrichment techniques using azinphos-methyl, malathion, or dimethioate as sole carbon sources. Cell-free crude enzyme preparations from the mixed cultures grown on dimethoate could hydrolyze this compound in addition to malathion and azinphos-methyl without any additional salt or co-factors being required.[58]

IV. CARBAMATES

A. Metabolism

The carbamates are a structurally and physiologically heterogeneous group of compounds. Their classification as carbamates is based upon the fact that they are all derivatives of carbamic acid (Figure 5). Some carbamates, such as carbaryl and carbofuran, act as insecticides, whose mode of action is that of inhibiting the enzyme acetylcholinesterase. Other carbamate compounds, such as chlorpropham and diallate, act as herbicides, which control plant growth by interfering with cell division, and additional classes of carbamates act as fungicides and nematocides.[59,60]

The N-methylcarbamates are plant systemic insecticides that are presently in wide-