Metabolism *in Vitro* of Diazinon and Diazoxon in Susceptible and Resistant Houseflies

Raymond S. H. Yang,[1] Ernest Hodgson, and W. C. Dauterman*

The metabolism of ethoxy-1-^{14}C-labeled diazinon and diazoxon was studied using microsomal and soluble enzyme preparations from susceptible and diazinon-resistant houseflies. Both diazinon and diazoxon are degraded by the microsomal mixed function oxidase system which requires NADPH and oxygen and is inhibited by carbon monoxide. Microsomes from the resistant housefly degrade diazinon and diazoxon to a greater extent than those from the susceptible housefly. This may account, in part, for the mechanism of diazinon resistance. The soluble fraction from the resistant housefly degrades both diazinon and diazoxon efficiently in the presence of reduced glutathione. The rate of diazinon degradation and the nature of the water soluble metabolites were studied.

The mechanism of organophosphate insecticide resistance in insects was believed to be largely due to detoxication by hydrolases and/or a difference in cuticular penetration of the insecticides (Jarczyk, 1966; O'Brien, 1967; Oppenoorth, 1965; Smith, 1962). Until recently, little was known concerning oxidation of various organic insecticides by insect microsomal preparations, and this area is under extensive investigation (Casida, 1969; Hodgson and Plapp, 1970; Hook *et al.*, 1968; Terriere, 1968; Wilkinson, 1968).

Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate] is an organophosphate insecticide to which many strains of houseflies (*Musca domestica*) have developed resistance (Brown, 1960; Forgash *et al.*, 1962; O'Brien, 1967). Forgash *et al.* (1962) reported that the mechanism of diazinon resistance in the housefly was due, in part, to a lower rate of penetration of the insecticide into the resistant housefly. This finding was confirmed later by Gwiazda and Lord (1967).

In an early study on the *in vitro* degradation of parathion and diazinon, Matsumura and Hogendijk (1964) showed that enzyme preparations from organophosphate-resistant (OP-resistant) houseflies hydrolyzed both parathion and diazinon to a greater extent than those from susceptible houseflies. This hydrolytic cleavage of organophosphate insecticides, presumably by hydrolases, was also demonstrated by Collins and Forgash (1970) in a recent study on the degradation of diazoxon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphate] by housefly homogenates.

The oxidative degradation of parathion in insects was first demonstrated by Nakatsugawa and Dahm (1967) using microsomal preparations from the American Cockroach (*Periplaneta americana*) fat body. In subsequent studies (Nakatsugawa *et al.*, 1968, 1969), this oxidative cleavage by microsomes from the housefly abdomen was also demonstrated with other organophosphate insecticides.

Department of Entomology, North Carolina State University, Raleigh, N.C. 27607

[1] Present address: Department of Entomology and Limnology, Cornell University, Ithaca, N.Y. 14850

Recently, ElBashir and Oppenoorth (1969) reported that the rates of formation and the subsequent degradation of diazoxon and paraoxon from the corresponding thiono compounds by housefly microsomal preparations were both much faster in OP-resistant houseflies than in susceptible ones. Later, Lewis (1969) and Lewis and Lord (1969) claimed that in several housefly strains a total of three mechanisms were responsible for diazinon resistance. These mechanisms involved the metabolism of diazinon and diazoxon by housefly microsomal and soluble enzyme preparations.

This study was undertaken to investigate the *in vitro* metabolism of diazinon and diazoxon in susceptible and resistant houseflies with special reference to oxidative degradation.

MATERIALS AND METHODS

Chemicals. Ethoxy-1-^{14}C-labeled diazinon and diazoxon were prepared, purified, and characterized as previously reported (Yang *et al.*, 1971). Pyrimidine ring labeled ^{14}C-diazinon (specific activity, 4.0 μCi/mg) was a gift from Giegy Chemical Corp., Ardsley, N.Y. Bovine serum albumin (BSA) fraction V was purchased from Armour Pharmaceutical Company, Chicago, Ill. The reduced forms of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH) were obtained from Sigma Chemical Co.

Insects. The diazinon-resistant houseflies were a gift from Dr. A. S. Perry of the National Communicable Disease Center, Savannah, Ga. This strain showed resistance to diazinon of 120-fold (topical LD_{50} 7.1 μg/female fly) and to diazoxon of 55-fold (topical LD_{50} 2.4 μg/female fly) when kept under diazinon (50 mg/ft^2) and malathion (200 mg/ft^2) stress. The Savannah strain was originally obtained from Dr. A. J. Forgash, Rutgers University. Susceptible flies of the CSMA strain were obtained from a culture originally supplied by Union Carbide Chemical Company and maintained at North Carolina State University since 1962. The resistant and susceptible strains of houseflies were maintained, as adults, on a diet of milk and sugar at 80° F and 55% R.H.

Enzyme Preparations. Adult houseflies (5–8 days old) were immobilized by carbon dioxide and retained on crushed

ice. The abdomens of female houseflies were removed with microdissecting scissors and then homogenized (10 female abdomens/ml) with a motor driven Teflon pestle in a fitted Pyrex tube in 0.15*M* potassium phosphate buffer, pH 7.8, containing 1% BSA. After filtering through a layer of cheesecloth, the crude homogenate was centrifuged at 12,000 g for 10 min. The resulting post-mitochondrial supernatant was filtered through glass wool and recentrifuged at 100,000 g for 1 hr. The microsomal pellet thus obtained was resuspended (70 female abdomens/ml) in 0.1*M* Tris-HCl buffer, pH 8.0, containing 2% BSA.

In the study on the subcellular localization of enzyme activity, female abdomens (20 female abdomens/ml) were homogenized directly in Tris buffer containing 1% BSA. The nuclear plus cell debris (600 g precipitate), mitochondrial (10,000 g precipitate), microsomal (100,000 g precipitate) and soluble (100,000 g supernatant) fractions were obtained by differential centrifugation. The pellets obtained from each centrifugation were resuspended in Tris buffer of the same concentration used for homogenization, at a ratio of 20 female abdomens/ml. One ml of each fraction was used in each incubation mixture. One gram of housefly abdomens was equivalent to approximately 80 abdomens (weight of abdomens varied from 10.41 mg–14.83 mg).

The enzyme preparations were prepared at 0° to 5° C and used immediately.

Incubation System. Unless otherwise stated, the incubation system consisted of: 120 μmoles Tris-HCl buffer, pH 8.0; 0.2 μmoles ^{14}C-diazinon or 0.1 μmoles ^{14}C-diazoxon; 1.1 μmoles NADPH and/or 2.0 μmoles GSH; microsomal preparation equivalent to 35 female abdomens; 0.5% BSA; distilled water to a final volume of 2.0 ml. The reaction mixture was incubated with shaking at 30° C for 1 hr. The subsequent assay method has been published elsewhere (Yang *et al.*, 1969), the essential feature being a partitioning of diazinon and diazoxon and their metabolites between benzene and water and the counting of the radioactivity of each phase.

Identification of Metabolites. The organosoluble components were separated and identified by the two phase paper chromatographic system (mobile phase: 50% dimethylformamide in water; immobile phase: 10% mineral oil in ether) Mitchell (1960). The radioactivity was detected by scanning the paper strips on a Packard Model 7201 Radiochromatogram Scanner. The diazinon standard was visualized with 0.5% 2,6-dibromoquinone-*N*-chloro-*p*-quinonimine in cyclohexane (Menn *et al.*, 1957), and the diazoxon standard was detected by the phosphorus reagent reported by Hanes and Isherwood (1949) using 1% $SnCl_2$ in methanol as the reducing agent.

The water soluble metabolites of diazinon and diazoxon were analyzed by the ion exchange chromatographic method of Plapp and Casida (1958a) using a 1.8 cm (i.d.) × 34 cm column of Dowex 1-X8 (50 to 100-mesh) anion exchange resin and a modified solvent system. The modified solvent system consisted of an initial stepwise elution and a subsequent gradient elution. The solvent system used was: (I) pH 3 HCl (200 ml); (II) pH 2 HCl (200 ml); (III) pH 1 HCl (200 ml); (IV) elution gradient (400 ml), pH 1 HCl plus methanol (1:3) to 1*N* HCl plus methanol (1:3); (V) elution gradient (400 ml), 1*N* HCl plus methanol (1:3) to concentrated HCl, H_2O and methanol (1:1:6). The authentic standards were detected by a phosphorus method described by Allen (1940) and the details of the method of separating and identifying the water soluble metabolites are described elsewhere (Yang *et al.*, 1971).

Table I. Degradation of Diazinon and Diazoxon in Subcellular Fractions of Housefly Abdomen Homogenates[a]

Fraction	Diazinon Degradation		Diazoxon Degradation[b]	
	Susceptible	Resistant	Susceptible	Resistant
Nuclei	0	3.0	5.9	3.9
Mitochondria	5.4	26.1	0	5.6
Microsomes	34.0	130.5	10.7	58.7
Soluble Fraction	0	3.8	0	4.8

[a] Enzyme preparation equivalent to 20 ♀ abdomens was added to each incubation. [b] The degradation is expressed as mμmoles/hr/g ♀ abdomen.

Table II. Effect of Reduced Glutathione and NADPH on the Degradation of Diazinon and Diazoxon by the Resistant Housefly Enzymes[a]

Enzyme Source	Cofactors	Diazinon[b] Degradation	Diazoxon[b] Degradation
Microsomes	None	0	0
	GSH	3.2	0
	NADPH	122.1	23.2
	NADPH + GSH	164.3	27.0
Soluble Fraction	None	52.3	5.2
	GSH	259.0	80.6
	NADPH	47.0	1.5
	NADPH + GSH	251.5	78.4

[a] Microsomes or soluble fraction equivalent to 35 or 10 ♀ abdomens, respectively, was added in each incubation. [b] The degradation is expressed as mμmoles/hr/g ♀ abdomen.

RESULTS AND DISCUSSION

Subcellular Distribution of Diazinon and Diazoxon-Degrading Enzymes. Centrifugal studies on housefly abdomen homogenates showed that both diazinon and diazoxon were degraded by a microsomal enzyme system in both resistant and susceptible houseflies (Table I).

There was a 4–5-fold difference between the degradation rates in the enzyme preparations from susceptible and resistant houseflies. The ability of the housefly microsomal enzymes to degrade diazoxon appeared to be much lower than their ability to degrade diazinon. Since the housefly diazinon and diazoxon-degrading enzymes appear to be of microsomal origin, the possibility that these reactions are the result of the microsomal mixed function oxidase system was investigated. Reduced pyridine nucleotide, NADPH, was found to be required for both diazinon and diazoxon degradation by the microsomal enzymes from both the susceptible and resistant houseflies. This was not the case with the rat. Diazoxon was degraded by a microsomal hydrolase while diazinon was degraded by mixed function oxidase system (Yang *et al.*, 1971).

Effect of Cofactors on the Degradation of Diazinon and Diazoxon. Lewis (1969) claimed that GSH-dependent desethylation reaction of both diazinon and diazoxon occurred in the soluble fraction from housefly homogenates. An attempt was made to verify these findings by comparing the effects of NADPH and GSH on the degradation of diazinon and diazoxon in the resistant housefly (Table II).

The findings demonstrate that the microsomal degradation of diazinon and diazoxon is oxidative in nature because of the requirement for NADPH. The effect of GSH on these reactions seemed to be insignificant. It may be noted, however, that the combined effect of GSH and NADPH on the micro-

Table III. Effect of Various Gases on the Degradation of Diazinon and Diazoxon

Gases Used	Diazinon Degradation[a]		Diazoxon Degradation[a]	
	Susceptible	Resistant	Susceptible	Resistant
Air—without bubbling	13.0	60.5	4.3	4.2
Air—with bubbling	13.1	76.5	4.1	9.6
Nitrogen	9.6	27.7	0	1.2
Carbon Monoxide	0.9	1.3	0	0

[a] Degradation rate expressed as mμmoles/30 min/g ♀ abdomen.

Table IV. Degradation of Diazinon and Diazoxon by Housefly Microsomes[a]

Compound	Degradation of ^{14}C-Compound (mμmoles/hr/g ♀ abdomen)	
	Susceptible	Resistant
^{14}C-Diazinon + Diazoxon	14.8	72.8
^{14}C-Diazinon	18.2	105.7
^{14}C-Diazoxon + Diazinon	0.1	0.9
^{14}C-Diazoxon	12.9	32.0

[a] 0.2 μmoles diazinon and/or 0.1 μmoles diazoxon added in each incubation.

somal enzymes was greater than additive. Whether or not GSH serves as a protective agent for the terminal oxidase (Ishikawa and Yamano, 1967) remains to be explored.

The findings also indicate that there is an efficient GSH-dependent enzyme system present in the soluble fraction of housefly homogenates which is responsible for the degradation of diazinon and diazoxon, substantiating the findings of Lewis (1969).

Comparison of the activity of the microsomal and soluble fractions can be made only if one assumes that the enzyme activity is directly proportional to the wet weight of the fly abdomens used. Due to the preparation procedure, the soluble enzyme preparation was equivalent to 10 female abdomens per assay, while the microsomal enzyme was 35 female abdomens per assay. This was taken into account for the calculations.

A certain amount of experimental variation occurred during each of the various experiments conducted. This was probably due to variability in the preparation of the insect microsomes. Very little quantitative data is available on the degradation of diazinon and diazoxon *in vitro* by insect preparations. However, the degradation of diazinon by our strain was approximately 2.5 times higher than that reported by Lewis (1969) for his strain of houseflies. Also, diazoxon degradation was approximately seven times higher than Lewis's findings, but 17 times less active than that reported by ElBashir and Oppenoorth (1969) for their resistant strains and approximately equal to their susceptible strains. In all cases the diazinon resistant strains were different and may account in part for the discrepancy between the three laboratories. However, Lewis used whole fly homogenates, and this could account for the lower degradation of diazinon and diazoxon since endogenous inhibitors found in the head and thorax will inhibit the mixed function oxidase system (Matthews and Hodgson, 1966; Tsukamoto and Casida, 1967). No explanation is available to account for the discrepancy between our findings and those of ElBashir and Oppenoorth. Differences in activity due to variations in preparative procedures seem unlikely for two reasons. As far as we can tell from the brief description of methods given by ElBashir and Oppenoorth (1969), the methods used were not radically different and, furthermore, a large number of comparisons of preparative methods for housefly microsomes have been carried out in this laboratory (Hansen and Hodgson, 1970).

Effect of Gases on the Degradation of Diazinon and Diazoxon. The methods used in the gas studies for diazinon and diazoxon degradation have been described previously (Yang *et al.*, 1969). As shown in Table III, the degradation of diazinon by housefly microsomes from both resistant and susceptible strains required oxygen. On the other hand, carbon monoxide strongly inhibits the reaction.

Although in the case of diazoxon degradation the enzyme activity was low compared to that with diazinon, the data seemed to indicate that the reaction, in the presence of various gases, followed the same pattern as that of diazinon degradation. As opposed to mammalian liver, in which diazoxon is not oxidized but hydrolyzed by a microsomal hydrolase system (Yang *et al.*, 1971), the degradation of diazoxon appears to be catalyzed slowly by the mixed function oxidase system. Since the mammalian hydrolase system is so much more efficient than the insect mixed function oxidase system in the degradation of diazoxon, this may account, in part, for the selectivity of diazinon.

Mixed Substrate Study. It is possible that both reactions are catalyzed by the same microsomal enzyme system in both resistant and susceptible houseflies. Therefore, a mixed substrate experiment was designed to investigate this possibility.

In one test, ^{14}C-diazinon was incubated with housefly microsomes and cofactors, and this was compared with the incubation of a mixture of ^{14}C-diazinon and nonlabeled diazoxon. Under the same condition, a similar experiment was performed by using ^{14}C-diazoxon and nonlabeled diazinon. Comparisons were made between the degradation of the ^{14}C-compound when incubated alone and with a nonlabeled counterpart. The results of this experiment are shown in Table IV.

It may be noted that the rate of degradation of ^{14}C-compounds was reduced when a nonlabeled counterpart was added. This effect was especially marked in the case of ^{14}C-diazoxon degradation. This correlates well with the observation that diazinon is a better substrate for the microsomal enzyme system and thus would be expected to compete more successfully for the site on the mixed function oxidases. Also the findings indicate that the same mixed function oxidase system is responsible for the degradation of both diazinon and diazoxon.

The Rate of Diazinon Degradation in Susceptible and Resistant Houseflies. In order to explain diazinon resistance in the housefly to diazinon in terms of differences in degradation, it is necessary to consider the time course of degradation. In other words, when insects are exposed to the insecticide, the amount of insecticide being degraded per unit time would be the determining factor in toxicity. Experiments were thus conducted on microsomal preparations from susceptible and resistant houseflies.

As shown in Figure 1, the resistant houseflies showed a more rapid rate of degradation than the susceptible strain. In view of the fact that under field conditions insects absorb the insecticide gradually, this difference in rate of degradation may account, in part, for the resistance of the housefly.

Nature of Metabolites. Since the enzymatic activities of the microsomal and soluble fractions from the susceptible

housefly were low, all studies on the characterization of metabolites were conducted on reaction mixtures with resistant housefly preparations.

A total of 24 analyses, 16 by paper chromatography of the organic phase and 8 by ion-exchange column chromatography of the aqueous phase, were carried out on the various reaction mixtures. The reaction mixtures were the result of experiments on the metabolism of diazinon and diazoxon by either microsomal or soluble enzymes in the presence of various cofactors (none, GSH, NADPH, NADPH + GSH). Each analysis was duplicated.

Organic extracts of the experiments shown in Table II were cochromatographed with the analytical standards. In the diazinon studies, diazinon was found to be the major radioactive compound in the organic phase in every case. However, when the reaction mixture contained microsomes and NADPH, significant levels of diazoxon in addition to diazinon were also detected. These findings suggest that diazinon is oxidized to diazoxon which, being slowly degraded by the microsomal mixed function oxidase system, accumulates in the reaction mixture. If diazinon is incubated with soluble housefly enzymes in the presence of GSH, the organic phase contains, in addition to diazinon, a minor metabolite (less than 1%) which has an R_f value very close to that of diazoxon. With the diazoxon studies, the only organic soluble compound detected in all cases was diazoxon.

Since the housefly microsomal and soluble fractions are inactive and no metabolism occurred unless suitable cofactors are provided (Table II), only eight analyses were made for the water soluble metabolites. These consist of the aqueous phases of diazinon and diazoxon degradation by housefly microsomes in the presence of NADPH or NADPH + GSH, and by the soluble housefly fractions in the presence of GSH or NADPH + GSH.

A typical ion-exchange chromatogram of the water soluble metabolites from microsomal degradation of diazinon is presented in Figure 2.

Peaks C and D were identified by cochromatography as diethyl phosphoric and diethyl phosphorothioic acids, respectively. Peak A appeared immediately after the column volume and these fractions were protein rich as indicated by uv absorbance at 280 nm and the Lowry protein test (Lowry *et al.*, 1951). Upon dialyzing the pooled fraction of peak A against distilled water for 48 hr, $^2/_3$ of the radioactivity still remained in the dialysis tube. These findings suggest that peak A might be a metabolite(s) which is bound to the protein. The identity of this (peak A) and also the minor peak B is still unknown. The ion-exchange chromatogram of the microsomal degradation products from diazinon in the presence of NADPH showed similar results.

Studies of diazoxon degradation by microsomal enzyme preparations with different cofactors (NADPH, NADPH + GSH) resulted in diethyl phosphoric acid as the major radioactive compound.

Figure 3 shows a typical ion-exchange chromatogram of the water soluble metabolites from the degradation of diazinon by the soluble fraction from a housefly homogenate.

Peaks C and D were again identified by cochromatography as diethyl phosphoric and diethyl phosphorothioic acids, respectively, and peak A the unknown protein bound metabolite.

It is interesting to note that in addition to the above mentioned metabolites, another major metabolite (peak B) was detected. The identity of peak B is still unknown, but based on the results obtained so far, the following characteristics

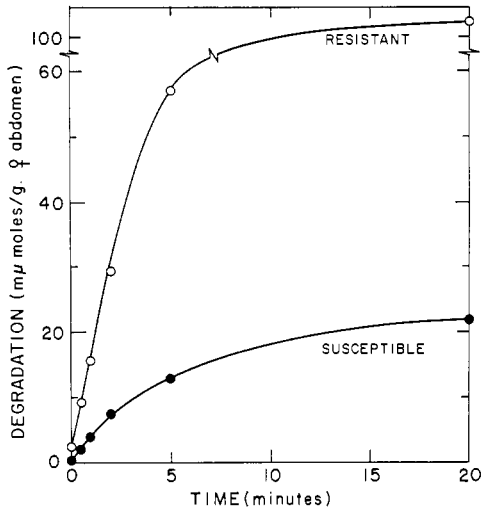

Figure 1. Rates of microsomal degradation of diazinon in susceptible and resistant houseflies

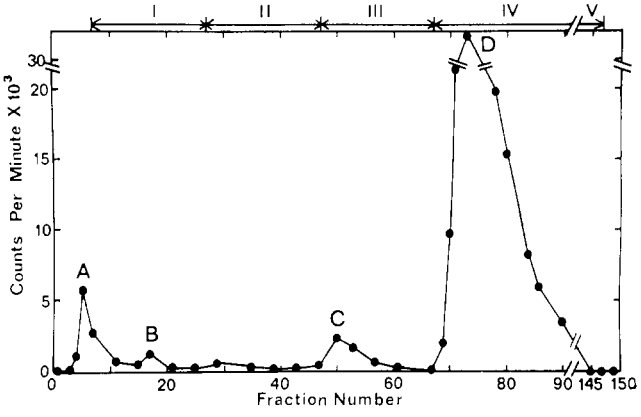

Figure 2. Ion-exchange chromatogram of water soluble metabolites of diazinon degradation by resistant housefly microsomes. Incubation mixture: Tris buffer, 1250 μmoles; ^{14}C-diazinon, 1 μmole; NADPH, 11 μmoles; GSH 20 μmoles; microsomes equivalent to 200 ♀ abdomens and distilled water added to a final volume of 14.5 ml. After two extractions with toluene, 12.1 ml of the aqueous phase was chromatographed. The Roman numerals shown on the top of the figure are in accordance with those of the solvent system mentioned in the text

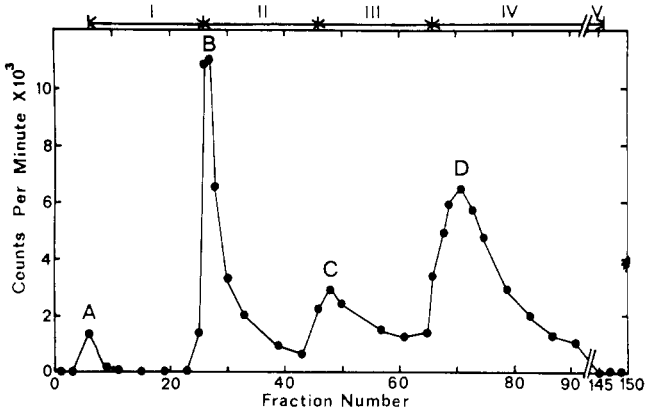

Figure 3. Ion-exchange chromatogram of water soluble metabolites of diazinon degradation by resistant housefly soluble enzymes. Incubation mixture: Tris buffer, 1250 μmoles; ^{14}C-diazinon, 0.8 μmoles; NADPH, 11 μmoles; GSH, 20 μmoles; soluble enzyme equivalent to 85 ♀ abdomens and distilled water added to a final volume of 14.5 ml. After two extractions with toluene, 17.6 ml (pooled aqueous phase of two tests) of the aqueous phase was chromatographed. The Roman numerals shown on the top of the figure are in accordance with those of the solvent system in the text

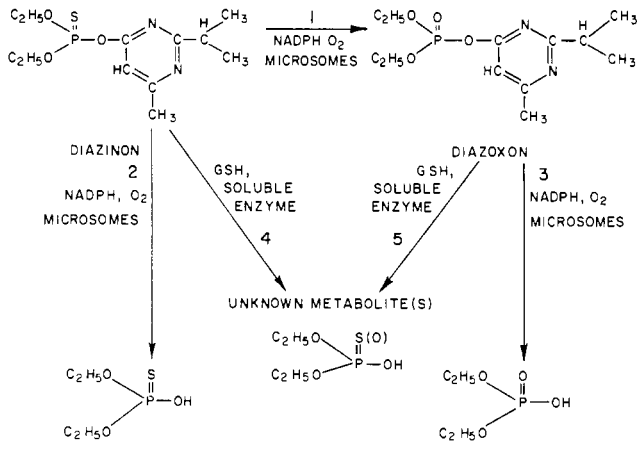

Figure 4. Proposed metabolic pathway of diazinon in the housefly

could be observed: (1) it is water soluble and is eluted from the ion-exchange column when HCl with pH 2 or lower is used as the solvent; (2) it is dialyzable; (3) it contains radioactivity from the ethoxy group; (4) it is neither diethyl phosphoric acid nor diethyl phosphorothioic acid; (5) diazoxon degradation by housefly soluble enzymes in the presence of GSH also gave rise to a peak in the same region as peak B of Figure 3; (6) using pyrimidine ring ^{14}C-labeled diazinon as the substrate, a similar peak at the identical position to peak B is detected, indicating the pyrimidinyl phosphate bond of the metabolite is intact. These findings and the fact that the reaction is catalyzed by a GSH-dependent soluble enzyme system suggest that peak B (Figure 3) contains desethyl diazinon. On the other hand, the possibility that the unknown metabolite (peak B in Figure 3) is desethyl diazinon is somewhat doubtful because of the following reasons: (1) the absence of another compound with equivalent radioactivity, presumably the GSH-bound ethyl group; (2) the previous reports show that the desalkyl products of organophosphates were eluted at a different position (elution gradient V of Figures 2 and 3) on a same type of ion-exchange column (Fukami and Shishido, 1966; Hollingworth *et al.*, 1967; Krueger *et al.*, 1959; Plapp and Casida, 1958b; Shishido and Fukami, 1963). Attempts to synthesize desethyl diazinon utilizing ethanolic KOH (Plapp and Casida, 1958b), and benzenethiol (Miller, 1962) were unsuccessful. No desethyl diazoxon was obtained with the NaI method (Spencer *et al.*, 1958). Since standard desethyl diazinon and desethyl diazoxon were not available, the identity of this metabolite (peak B) was difficult to resolve.

Recently, a new metabolite, hydroxydiazinon {*O,O*-diethyl *O*-[2-(2′-hydroxy-2′-propyl)-4-methyl-6-pyrimidinyl] phosphorothioate} was shown to occur on field-sprayed kale (Pardue *et al.*, 1970). The possibility of peak B (Figure 3) containing a ring hydroxylated compound remains to be investigated. Further work on the characterization of this metabolite is in progress.

In the case of diazoxon degradation by the soluble housefly enzyme, the ion-exchange chromatograms showed two major metabolites, an unknown metabolite (in the region of peak B) and diethyl phosphoric acid (peak C), and a minor one (peak A).

DISCUSSION

Based on these and previous findings (ElBashir and Oppenoorth, 1969; Oppenoorth and Van Asperen, 1961; Forgash *et al.*, 1962; Lewis, 1969) four factors are involved in diazinon resistance in the housefly. The first is the mode of entry of the insecticide, the second is the oxidative degradation in the microsomes, the third hydrolytic degradation of oxo-compounds, and the fourth, metabolism by a GSH-dependent soluble system. All four factors seem to be involved in the differences between susceptible and resistant houseflies. This observation confirms the preliminary findings from our laboratory that the microsomal oxidative activities (diazinon degradation, *N*- and *O*-demethylation), NADPH oxidation, O_2 uptake and cytochrome P_{450} level are all higher in diazinon resistant houseflies than in susceptible houseflies (Folsom *et al.*, 1970).

Summarizing the information concerning diazinon metabolism in the housefly, a metabolic pathway (Figure 4) could be presented. Once diazinon enters the body of the insect, it is subjected to several enzymatic reactions.

First, diazinon is activated to diazoxon (reaction 1), a more potent cholinesterase inhibitor, to exert its toxic action. This activation is catalyzed by the microsomal mixed function oxidase system (ElBashir and Oppenoorth, 1969). Meanwhile, the same microsomal mixed function oxidase system degrades diazinon (reaction 2) and diazoxon (reaction 3) to diethyl phosphorothioic and diethyl phosphoric acids, respectively. Whether the pyrimidine moiety undergoes hydroxylation reactions as in the case of the rat (Mücke *et al.*, 1970) is uncertain.

In addition to the microsomal oxidation reactions, diazinon and diazoxon are also degraded rapidly by a GSH-dependent soluble enzyme system (reactions 4 and 5). The identity and the fate of the metabolites(s) are under investigation. Furthermore, diethyl phosphorothioic and/or diethyl phosphoric acids are also produced under these conditions. The mechanism for formation of these metabolites in the soluble fraction is not known.

All three microsomal oxidations (reactions 1, 2, and 3) and the two GSH-dependent reactions (reactions 4 and 5) have been shown to occur in the susceptible housefly at a much lower rate (ElBashir and Oppenoorth, 1969; Lewis, 1969). Presumably, the relationship between the rate of detoxication (reactions 2, 3, 4, and 5) and the rate of intoxication (reaction 1) determines the toxic effect to the insects.

ACKNOWLEDGMENT

The technical assistance of Loretta Hsieh and Frances Penny is greatly appreciated.

LITERATURE CITED

Allen, R. J. L., *Biochem. J.* **34,** 858 (1940).
Brown, A. W. A., *Ann. Rev. Entomol.* **5,** 301 (1960).
Casida, J. E., "Insect Microsomes and Insecticide Chemical Oxidations," in Microsomes and Drug Oxidations, J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, G. J. Mannering, Eds., pp. 517–31, Academic Press, New York, 1969.
Collins, W. J., Forgash, A. J., *J. Econ. Entomol.* **63,** 394 (1970).
ElBashir, S., Oppenoorth, F. J., *Nature* (London) **223,** 210 (1969).
Folsom, M. D., Hansen, L. G., Philpot, R. M., Yang, R. S. H., Dauterman, W. C., Hodgson, E., *Life Sci.* **9,** 869 (1970).
Forgash, A. J., Cook, B. J., Riley, R. C., *J. Econ. Entomol.* **55,** 544 (1962).
Fukami, J., Shishido, T., *J. Econ. Entomol.* **59,** 1338 (1966).
Gwiazda, M., Lord, K. A., *Ann. Appl. Biol.* **59,** 221 (1967).
Hanes, C. S., Isherwood, F. A., *Nature* (London) **164,** 1107 (1949).
Hansen, L. G., Hodgson, E., *Biochem. Pharmacol.* submitted for publication (1970).
Hodgson, E., Plapp, F. W., J. AGR. FOOD CHEM. **18,** 1048 (1970).
Hollingworth, R. M., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **15,** 242 (1967).
Hook, G. E. R., Jordan, T. W., Smith, J. N., "Factors Affecting Insect Microsomal Oxidations," in The Enzymatic Oxidations of Toxicants, E. Hodgson, Ed., pp. 27–46, North Carolina State University at Raleigh, 1968.

Ishikawa, Y., Yamano, T., *Biochim. Biophys. Acta* **131,** 490 (1967).
Jarczyk, H. J., *Pflanzenschutz-Nachr. Bayer* **19,** 1 (1966).
Krueger, H. R., Casida, J. E., Niedermeier, R. P., J. AGR. FOOD CHEM. **7,** 182 (1959).
Lewis, J. B., *Nature* (London) **224,** 917 (1969).
Lewis, J. B., Lord, K. A., *Proc. 5th Br. Insect. Fung. Conf.*, **465** (1969).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randell, R. J., *J. Biol. Chem.* **193,** 265 (1951).
Matsumura, F., Hogendijk, C. J., J. AGR. FOOD CHEM. **12,** 447 (1964).
Matthews, H. B., Hodgson, E., *J. Econ. Entomol.* **59,** 1286 (1966).
Menn, J. J., Erwin, W. F., Gordon, H. T., J. AGR. FOOD CHEM. **5,** 601 (1957).
Miller, B., *Proc. Chem. Soc.*, p. 303, 1962.
Mitchell, L. C., *J. Ass. Offic. Agr. Chem.* **43,** 810 (1960).
Mücke, W., Alt, K. O., Esser, H. O., J. AGR. FOOD CHEM. **18,** 208 (1970).
Nakatsugawa, T., Dahm, P. A., *Biochem. Pharmacol.* **16,** 25 (1967).
Nakatsugawa, T., Tolman, N. M., Dahm, P. A., *Biochem. Pharmacol.* **17,** 1517 (1968).
Nakatsugawa, T., Tolman, N. M., Dahm, P. A., *J. Econ. Entomol.* **62,** 408 (1969).
O'Brien, R. D., "Insecticides, Action and Metabolism," Academic Press, New York, 332 pp., 1967.
Oppenoorth, F. J., Van Asperen, K., *Entomol. Exp. Appl.* **4,** 311 (1961).
Oppenoorth, F. J., *Ann. Rev. Entomol.* **10,** 185 (1965).
Pardue, J. R., Hansen, E. A., Barron, R. P., Chen, J. T., J. AGR. FOOD CHEM. **18,** 405 (1970).
Plapp, F. W., Casida, J. E., *Anal. Chem.* **30,** 1622 (1958a).
Plapp, F. W., Casida, J. E., *J. Econ. Entomol.* **51,** 800 (1958b).
Shishido, T., Fukami, J., *Botyu-Kagaku* **28,** 69 (1963).
Smith, J. N., *Ann. Rev. Entomol.* **7,** 465 (1962).
Spencer, E. Y., Todd, A., Webb, R. F., *J. Chem. Soc.* 2968 (1958).
Terriere, L. C., *Ann. Rev. Entomol.* **13,** 75 (1968).
Tsukamoto, N., Casida, J. E., *Nature* (London) **213,** 49 (1967).
Wilkinson, C. F., "Detoxication of Pesticides and the Mechanism of Synergist," in The Enzymatic Oxidations of Toxicants, E. Hodgson, Ed., pp. 113–49, North Carolina State University at Raleigh, 1968.
Yang, R. S. H., Dauterman, W. C., Hodgson, E., *Life Sci.* **8,** 667 (1969).
Yang, R. S. H., Hodgson, E., Dauterman, W. C., J. AGR. FOOD CHEM. in press (1971).

Received for review July 21, 1970. Accepted October 26, 1970. Paper number 3242 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, N.C. Work supported in part by Grant No. ES-00044 from the U.S. Public Health Service.