AN ABSTRACT OF THE THESIS OF

CHARLES ROBERT WALKER (Name) for the MASTER OF SCIENCE (Degree)

in ENTOMOLOGY (Major) presented on Sept 4, 1969 (Date)

Title: INDUCTION OF MICROSOMAL OXIDASES BY DIELDRIN IN HOUSE FLIES

Abstract approved: Redacted for Privacy

L. C. Terriere

Previous investigations have shown that exposure to DDT results in increased activity in soluble and microsomal enzyme in insects. These responses in DDT-resistant Triatoma infestans nymphs and in adults of DDT-resistant Musca domestica (the common house fly) are accompanied by increased RNA and protein synthesis. The phenomenon has been termed induction. Since induction has not been demonstrated in insecticide susceptible insects, the phenomenon is thought to be associated with resistance. The research reported here was a study of the inductive effect of two other chlorinated organic insecticides on house flies and the relationship between induction and insecticide resistance.

The house fly strains studied were resistant to dieldrin and thus could tolerate this material and its precursor, aldrin, when these were applied as inducers. The strains differ in microsomal

oxidase activity with Isolan-R highest, Orlando intermediate, and dield; cyw lowest.

The flies were treated with inducers by two methods, by 24 hour exposure to deposits of test compounds in glass jars and by topical application of the compounds. The response to the inducers was measured by in vivo and in vitro methods. Mortality tests with the insecticide carbaryl, indicated the in vivo response. Assays of naphthalene hydroxylase and heptachlor epoxidase were used to measure the effect of the inducers on microsomal enzyme activity.

The effect of the treatments on protein synthesis was determined by use of a protein synthesis inhibitor, cycloheximide. Incorporation of C^{14}-isoleucine into subcellular protein was also followed.

Aldrin and dieldrin caused a large increase in microsomal oxidase activity and an increased tolerance to carbaryl. The response was dose dependent. The age of the flies affects the magnitude of response but not the potential to respond.

Cycloheximide inhibited both in vivo and in vitro responses to dieldrin. Dieldrin also caused an increased incorporation of isoleucine into microsomal and soluble protein. Additional experiments showed that dieldrin does not stimulate enzyme activity when added during various stages of microsome preparation. BSA, which is reported to neutralize an endogenous inhibitor in microsome

preparation, produced an additive effect with dieldrin treated flies. Kinetic studies revealed that dieldrin effects only the amount of oxidase present.

It was concluded that aldrin and dieldrin exerted their effect in house flies through increased synthesis of protein. The inductive action of dieldrin in house fly strains appears to depend on the levels of their microsomal oxidase activity, being greatest when microsomes are most active. This suggests that the action of the inducer is at the site of gene regulation of protein synthesis, and results in increased production of detoxication enzymes.

Induction of Microsomal Oxidases by Dieldrin in House Flies

by

Charles Robert Walker

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1970

APPROVED:

Redacted for Privacy

Sept 10, 1969

Professor of Insect Toxicology
in charge of major

Redacted for Privacy

Chairman of Department of Entomology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 9-4-69

Typed by Donna L. Olson for Charles Robert Walker

ACKNOWLEDGMENT

I wish to express my sincere appreciation to Dr. L. C. Terriere for his assistance throughout this study.

I would also like to thank Dr. F. W. Plapp, Jr. for the valuable suggestions he made in the beginning of this investigation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INDUCTION OF MICROSOMAL OXIDASES BY DIELDRIN IN HOUSE FLIES

INTRODUCTION AND LITERATURE REVIEW

Many compounds are known, which, on application to vertebrates, cause increased activity in the drug metabolizing systems of the liver. One group of compounds, the chlorinated organic insecticides, has been the subject of considerable research in vertebrates. Their effect on soluble and microsomal enzymes is thought to be due to a change in the genetic control of protein synthesis and has been termed induction.

Another phenomenon, the development of resistance to insecticides by insects, is also thought to involve microsomal enzymes (Agosin, et al., 1961; Schonbrod, Philleo and Terriere, 1965; Tsukamato and Casida, 1967; Oppenoorth, 1967). These investigators have reported increased levels of microsomal oxidase activity in resistant insects. Additional research revealed that DDT has an effect on the total contents of NADP (an essential component of drug metabolizing systems) in DDT-resistant nymphs of Triatoma infestans (Agosin and Dinamarca, 1963; Agosin, Scarmelli, Dinamarca and Aravena, 1963). This increase in NADP was attributed to an increase in NAD kinase resulting from induction (Ilivicky,

Dinamarca and Agosin, 1964). These workers speculated that resistance to DDT might be the result of increased production of NADP by induced NAD kinase. Two questions were raised by Agosin's group; is the DDT effect a matter of induction, and does induction play a role in resistance?

Further research disclosed that male Triatoma infestans incorporated 1-C^{14}-glycine into protein at a slower rate than nymphs when both were treated with DDT (Ilivicky and Agosin, 1967). NAD kinase from Triatoma infestans was purified, and a specific antiserum prepared against the enzyme. This enabled Agosin, Ilivicky and Litvak (1967), to show the de novo synthesis of an enzyme in DDT-resistant nymphs previously treated with DDT.

In work with house flies, Agosin et al. (1966), found that DDT treatment of a resistant strain stimulated the pentose phosphate pathway, increased the available NADP, and enhanced the rate of protein synthesis. These effects were not observed in a susceptible strain. Further information on the role of induction in resistance was reported by Gil et al. (1968) who treated DDT-resistant and susceptible flies with DDT and found that DDT induced microsomal enzymes only in the resistant strains. They also reported that this induction was accompanied by an increase in the rate of incorporation of C^{14}-uracil into RNA.

Other investigations supporting induction by DDT in house flies

and nymphs of Triatoma infestans have shown an increased incorporation of labeled amino acids in nymphal Triatoma infestans (Agosin, Aravena and Neghme, 1964). Another study illustrated an increased in vivo and in vitro incorporation of C^{14}-labeled amino acids into polysomal proteins of DDT-resistant house fly strains (Litvak and Agosin, 1968b). A third group of studies showed increased ribonucleic acid metabolism in resistant house fly strains (Balazs and Agosin, 1968) and nymphal Triatoma infestans (Litvak et al., 1968a). The last investigators, noting that DDT causes induction only in resistant insects, suggested that current views on the mechanism of resistance to DDT may have to be modified in that, not only selection, but also enzyme induction, mediated through the DNA-dependent synthesis of RNA, plays a role.

One investigator (Morello, 1964) reported that microsomes from Triatoma infestans hydroxylated DDT and that 3-methylcholanthrene increased DDT metabolism and reduced mortality. This has been the only report of induction in insects involving a compound other than DDT.

Not all attempts to demonstrate induction in insects have been successful. Meksongsee, Yang and Guthrie (1967) tested mortality of DDT-resistant and susceptible house flies to carbamate insecticides after DDT or phenobarbital feedings, and found no significant decrease in toxicity of the carbamates. Chakroborty and Smith

(1967) were unable to detect an increase in the oxidation of alkylbenzenes in locusts after pretreatment with phenobarbitone or 3,4-benzopyrene. Finally, Oppenoorth and Houx (1968), using one of the DDT-resistant house fly strains, Fc, investigated by Gil et al. (1968), could detect no increase in DDT metabolism after DDT pretreatment. It may be significant that the amount of DDT used by Oppenoorth and Houx was a dose shown by Gil to reduce the activity of the microsomal enzymes. Thus the negative results of Oppenoorth do not necessarily disagree with results of Gil.

The previous investigators have proven that DDT does induce enzyme synthesis in insects. They have also shown a relationship between the potential to be induced and resistance. However, the role of induction in the development of resistance has not been substantiated.

As both mammals and insects repond similarly to DDT, it is probable that other chlorinated insecticides cause responses in insects similar to those evidenced in mammals. Therefore, the objectives of this investigation were to measure the inductive effect of two other chlorinated insecticides, aldrin and dieldrin, on house flies; to test Litvak's theory about the role of induction in resistance; and to further characterize the phenomenon of induction.

MATERIALS AND METHODS

House Fly Strains Studied

Dieldrin-R Curly Wing (dield; cyw)

Obtained from the USDA laboratory, Corvallis. Selected for dieldrin resistance, susceptible to non-cyclodiene insecticides and low in microsomal oxidase activity (Schonbrod, et al., 1968).

Orlando DDT (Orlando)

Maintained for ten years in our laboratory. Cross-resistant to dieldrin and intermediate in microsomal oxidase activity.

Isolan-R

Obtained from the USDA laboratory, Corvallis. Selected for resistance to the carbamate, Isolan. Resistant to dieldrin and organophosphates. High in microsomal oxidase activity.

Treatment of House Flies with Inducing Compounds

Two methods were used to treat the flies with sublethal doses of the stimulating insecticides. Groups of 100 flies were placed in pint jars precoated with the insecticide. During the treatment period they were kept at 70°F. and had access to water and a 6: 6: 1 mixture

of sugar, powdered non-fat milk, and powdered egg yolk.

The second method of exposure was by topical drop, applying one microliter of an acetone solution to the abdomen. During the treatment period, the flies were placed in small cages with food and water. Colony flies held in regular rearing cages served as controls.

Susceptibility of Treated Insects to Carbaryl, Mobam and Naphthalene

Mortality response to carbaryl and mobam was measured by placing 25 flies in pretreated pint jars. Temperature was 70°F. The flies were given water and were checked for mortality at 24 hours. The dose range causing 90 to 100 percent mortality in non-induced flies was used. Specific dose information is given in the results section.

The mortality response to naphthalene vapors was conducted as described by Schafer (1969). Comparisons between treated and colony flies were in terms of LT_{90}, the exposure time giving 90 percent mortality.

Treatment with Cycloheximide and UL-C^{14}-isoleucine

Flies were immobilized by cold and injected in the thorax with one microliter of an aqueous solution using a 27 gauge needle. The

specific doses for the two compounds are given in the results section. Neither treatment caused mortality in the flies.

Enzyme Assays

The amount of induction was determined by measuring the increase in microsomal hydroxylase or epoxidase activity. Microsomes were prepared by the methods of Schonbrod, Philleo, and Terriere (1965). The incubations were generally for periods of 15 or 30 minutes with microsomal equivalents of 25 or 50 flies. Two substrates were used, 1-C^{14}-naphthalene (250 mμmoles), and heptachlor (100 mμmoles). The enzyme reactions were stopped with 15 ml of ethyl ether for the former and with 15 ml of a 3:2 hexane:2-propanol solution for the latter.

The amount of naphthalene hydroxylated was determined by pipetting a 0.2 ml portion of the ether phase onto 3.8 x 2.0 cm paper strips (Whatman No. 4), allowing to dry in a hood for seven minutes to permit loss of unreacted naphthalene. The strips were then placed in 15 ml of dioxane counting solution in a Nuclear Chicago scintillation counter and counted. The aqueous phase of the incubates was assayed by pipetting 0.1 ml on a strip and drying for 12 minutes before counting.

The heptachlor incubates were extracted by shaking twice with 20 ml portions of hexane. They were analyzed by gas liquid

chromatography. Calculations were based on peak heights obtained with standard solutions.

Test of Protein Synthesis

The effect of the inducer on amino acid incorporation into protein was determined for the mitochondrial, microsomal, and soluble fraction. Flies were injected with 0.01 or 0.02 uC of UL-C^{14}-isoleucine and the incorporation allowed to proceed for 24 hours. The mitochondrial and microsomal fractions were resuspended in pH 7.8 phosphate buffer before addition of an equal volume of ice cold 10% TCA. The precipitates were washed once with ice cold 10% TCA and dissolved in 1 N NaOH by heating at 100°C. for five minutes. A portion of each solution was used for protein determination by the Biuret method. The rest was placed in a scintillation vial with 10 ml of a toluene counting solution. One hundred percent ethanol was then added to solubilize the aqueous phase and the vials were counted. A correction factor for quenching was determined by use of C^{14}-toluene (6660 dpm) as an internal standard.

RESULTS AND DISCUSSION

Confirmation of Induction

Preliminary experiments with the Orlando strain indicated that aldrin and dieldrin treatment caused increased microsomal oxidase activity. Results of a typical experiment are seen in Table I which shows that the treatments resulted in a 2-3 fold increase in microsomal hydroxylase activity. Other preliminary experiments included estimation of a significance value for enzyme stimulation, investigation of stress effects encountered with jar held flies, determination of the effects of dieldrin when added at various stages of microsome preparation, and consideration of the role of endogenous inhibitors on dieldrin stimulation. Further experiments were designed to indicate the characteristics of the dieldrin effect on microsomal oxidase activity, and to establish whether it involved increased synthesis of protein.

Degree of Activation

The precision of the enzyme assays and the minimal activation required for significance was established in experiments with groups of identically reared flies. Six groups consisting of twenty-five four day old male flies from the colony cage, and six similar groups held in jars treated with 50 μg of dieldrin, were assayed for microsomal

Table I. Naphthalene hydroxylase activity of microsomes of Orlando house flies exposed to aldrin or dieldrin for 24 and 48 hours.

Insecticide Treatments[2]	Naphthalene hydroxylation products μμ moles/fly/min.[1]			
	24 hours		48 hours	
	♀	♂	♀	♀
Untreated colony	11.3	17.3	17.4	19.0
Untreated jar	9.9	14.4	--	--
Aldrin 75 μg/jar	--	52.0	--	30.0
Aldrin 100 μg/jar	22.0	--	23.0	--
Dieldrin 50 μg/jar	47.0	44.0	35.0	32.0

[1] Incubation of 50 fly equivalents for 15 minutes, average of duplicate determinations.

[2] Flies two days old at beginning of experiment, exposed at 72°F.

epoxidase activity. The values for the colony flies ranged from 124.0 to 149.0 μμmoles heptachlor epoxide/fly, with an average of 137.0 and a mean difference of 12. Epoxide production by microsomes of the dieldrin treated flies ranged from 337.0 to 398.0 μμmoles/fly and averaged 370.0 with a mean difference of 30 μμmoles/fly. The change in enzyme activity due to the dieldrin treatment averaged 233 μμmoles product/fly. From these values, the assumption was made that increases in epoxidase activity resulting in the production of more than 60 μμmoles epoxide were of significance.

Effect of Stress

Preliminary enzyme assays comparing colony and dieldrin-treated flies always showed a large increase after dieldrin treatment. However, holding control flies in untreated jars also exerted some effect on enzyme activity, the amount depending on the temperature. It was found that this effect was minimized by conducting the treatments at 70°F. The magnitude of this jar stress effect is seen in Table I. The effect of this stress on dieldrin-treated flies was measured by comparing flies treated with dieldrin in jars with flies treated topically, then held in cages. The response after 24 hours was similar for both treatments. It was for this reason that flies treated by the jar method were compared to caged colony flies rather

than jar held flies.

Dieldrin-microsome Interaction

The possibility that dieldrin had some activating effect on fly homogenates was tested by adding dieldrin at various stages during the preparation of microsomes then measuring their naphthalene hydroxylase activity. Eleven micrograms of dieldrin in 0.1 ml of methylcellusolve was added to the homogenate of the colony flies. GLC analysis had shown that this amount of dieldrin was present in homogenates of flies given a 24 hour exposure to 50 μg of dieldrin. This concentration of dieldrin had no effect on microsomal hydroxylase activity as compared to untreated homogenates.

Dieldrin was also added to the mitochondrial supernatant at 1, 10, 20, 50, and 100 μg, and the mixture centrifuged as usual. Microsomal preparations from female Orlando flies of two different ages, both with and without dieldrin pretreatment in jars, were unaffected by this treatment. The addition of dieldrin to the resuspended microsomes also had no effect on enzyme activity. From these experiments it was concluded that the increased microsomal activity observed in dieldrin pretreated flies was not due to dieldrin-microsomal interaction during preparation.

Role of Endogenous Inhibitor

Bovine serum albumin (BSA), has been used by several investigators (Tsukamoto and Casida, 1967; Schonbrod, personal communication), to increase the activity of microsomal oxidase in house flies. The effect of BSA is presumed to be the neutralization of an endogenous inhibitor of microsomal enzymes. To determine whether dieldrin treatment had a similar effect, BSA was included in a typical dieldrin activation experiment. Five day old females of the dield; cyw, Orlando, and Isolan-R strains were treated with 50 μg of dieldrin per jar for 24 hours and used for enzyme determinations along with colony flies. Heptachlor epoxidation was measured both with and without BSA addition to the incubates.

The results indicated that the efects of BSA and dieldrin were additive in all three strains (Table II). This is interpreted as evidence that dieldrin stimulates activity by a different mechanism than does BSA.

Kinetic Studies on Microsomal Oxidase

Two additional ways which an enzyme can be characterized are by plotting velocity progress curves, and by measuring Km and Vmax values (Dixon and Webb, 1964).

The rate of heptachlor epoxidation by microsomes of Orlando

Table II. Effect of BSA on microsomal heptachlor epoxidase activity of female dield;cyw, Orlando, and Isolan-R house flies treated with dieldrin.

Dieldrin Treatment[2]	BSA[3]	Heptachlor epoxide μμmoles/fly[1]		
		Dield; cyw	Orlando	Isolan-R
Untreated	untreated	122	47	319
Untreated	1 mg/fly	263	137	623
50 μg	untreated	400	411	598
50 μg	1 mg/fly	624	710	955

[1] Incubation of 25 fly equivalents for 30 minutes, average of duplicate samples.

[2] Flies were five days old when treated.

[3] BSA was added to the microsomal incubates.

Table III. Lineweaver-Burk analysis of the heptachlor oxidase of Orlando colony and dieldrin treated house flies.[1]

Treatment	Km μμ molar		V_{max} 10^{-13} moles product/fly/min.	
	♀	♂	♀	♂
Untreated colony	18.1	16.6	4.16	2.80
Dieldrin[2]	14.2	14.2	16.6	25.0

[1] Three day old flies, microsome equivalents of ten flies.

[2] 50 μg dieldrin per jar for 24 hours.

colony and dieldrin treated flies (50 μg/jar for 24 hours) of both sexes was determined. Duplicate groups of three day old flies were homogenized as usual. Ten fly microsomal equivalents were incubated for 0, 5, 10, 15, 30, and 60 minutes and the μμmoles product formed per fly was plotted against time (Figure 1). This shows that the rate of heptachlor epoxidation in dieldrin treated flies is greater than in colony flies, indicating a more active enzyme system in the treated flies.

Lineweaver-Burk plots (Figure 2) of heptachlor epoxidation by microsomes of three day old Orlando flies of both sexes were made for both colony and dieldrin treated flies (50 μg/jar for 24 hours). Ten fly microsomal equivalents were incubated, in duplicate, for 30 minutes with 25, 50, 100, 200, and 400 mμ molar heptachlor. The Km and Vmax values were read from the plots and are shown in Table III.

The Km values indicate the affinity of the enzyme for the substrate, and are the same for colony and dieldrin treated flies. This indicates that the difference between colony and dieldrin-treated flies is not a change in the enzyme. The Vmax values, which represent the rate of the reaction, show that the dieldrin treated flies have a more active enzyme system than the colony flies. These results suggest that dieldrin results in an increase in the amount of functional enzyme.

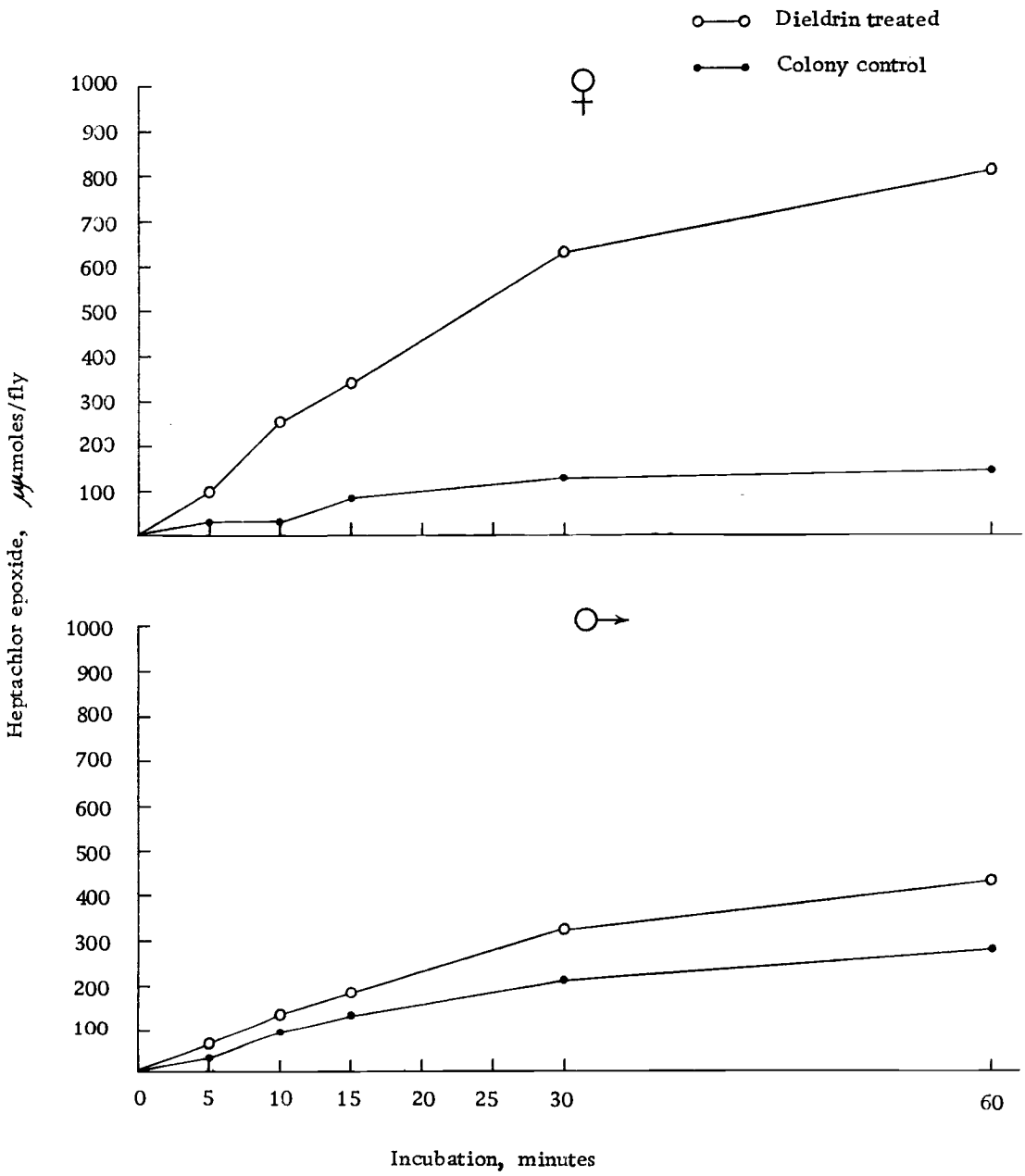


Figure 1. Rate of production of heptachlor epoxide by microsomes of Orlando house flies treated with dieldrin. Four day old female and five day old males exposed to dieldrin at 50 μg per jar.

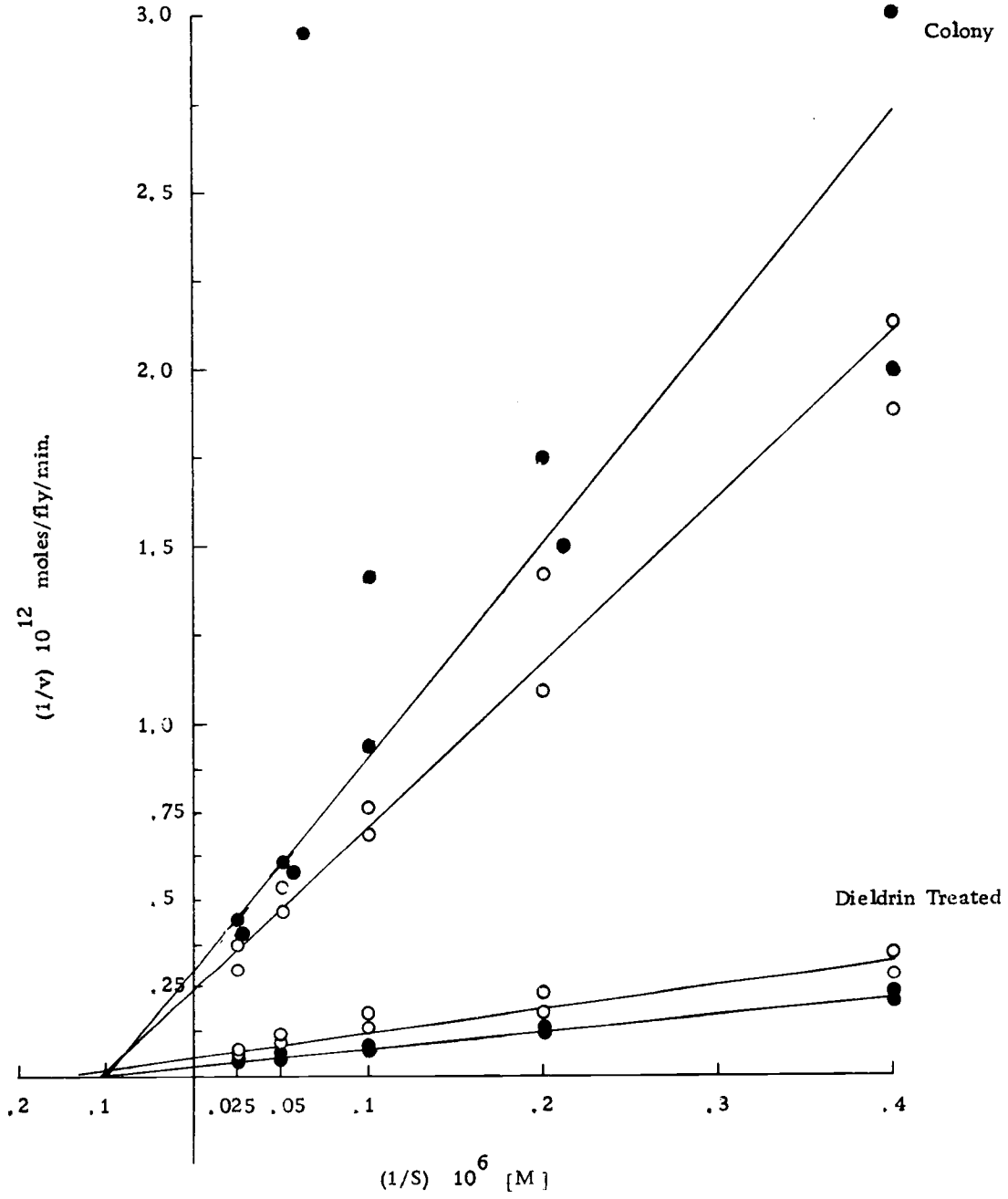


Figure 2. Lineweaver-Burk plots of microsomal heptachlor epoxidase of Orlando house flies treated with 50 μg of dieldrin per jar. Flies were three days old, ten fly equivalence per incubate. ● = Male; o = Female.

Effect of Dieldrin on Protein Synthesis

Cycloheximide inhibits protein synthesis by interfering with peptide bonding at the ribosomal complex (Ennis and Lubin, 1964). It was of interest to determine the effect of this drug, in combination with dieldrin, on house fly microsomal oxidase activity and on mortality tests.

Tests revealed that 1 μg of cycloheximide injected into the flies produced no mortality after 48 hours. Cycloheximide did not sensitize colony flies to carbaryl.

Three experiments were conducted using flies from different generations. In two experiments, Orlando males were injected with 1 μg of cycloheximide, then exposed to 50 μg of dieldrin in precoated jars. The first experiment was with four day old flies and the second six day old flies. Controls were injected with either 1 μl of water or with an empty needle (for the wound effect). Additional controls during the incubations included microsomes of untreated colony flies and flies treated only with dieldrin. After 24 hours at 70°F. the flies were removed from the jars and homogenized for microsome preparation. The extent of naphthalene hydroxylation indicated the activity of the enzymes (Table IV).

No increase in naphthalene hydroxylase activity was exhibited by microsomes from the flies treated with both cycloheximide and

Table IV. Microsomal naphthalene hydroxylation and mortality due to carbaryl in Orlando male house flies treated with cycloheximide before exposure to dieldrin.[1]

Treatment	Naphthalene Hydroxylation Products μμM/fly/min. expt. 1[2]	Naphthalene Hydroxylation Products μμM/fly/min. expt. 2[3]	Percent Mortality after 24 hour exposure to carbaryl[4]
Untreated colony	9.12	10.54	85
Dieldrin	23.67	24.40	34
Injected[5]	7.65	--	--
Injected + Dieldrin[5]	24.55	--	--
Water Injected	7.60	8.86	--
Water Injected + Dieldrin	13.42	16.82	38
Cycloheximide Injected	9.86	6.08	--
Cycloheximide Injected + Dieldrin	9.37	9.71	95

[1] 50 μg/jar, two generations of flies tested.

[2] Flies were four days old.

[3] Flies were six days old.

[4] Twenty-five flies and 5 mg carbaryl/jar, average of four replicates.

[5] Insects punctured in thoracic region with empty needle.

dieldrin, while dieldrin treatment alone resulted in a 160% increase in one experiment and a 130% increase in the second. Injection with water showed an inhibitory effect on naphthalene hydroxylase activity but was not the result of protein synthesis inhibition as is shown by further studies on amino acid incorporation experiments (Table VI).

Separate groups of flies were given the same treatments with cycloheximide and dieldrin as in the previous experiments, and then assayed for susceptibility to carbaryl. These results are also shown in Table IV. The increased tolerance to carbaryl in flies pre-treated with dieldrin was prevented by cycloheximide. Eighty-five percent of the colony flies died, compared with 34% of the dieldrin treated and 95% of the cycloheximide-dieldrin treated flies. A 20% variation in mortality occurs with carbaryl tests.

In the third experiment three day old Orlando flies were injected with 1 μg of cycloheximide and then exposed to three different levels of dieldrin, 50, 100, and 200 μg per jar. Cycloheximide was also added to the homogenates of two samples to determine the effect on oxidase activity. The microsomes were then assayed for heptachlor epoxidase activity. During the analysis of heptachlor epoxide, the dieldrin level in the microsomes was also measured to determine whether cycloheximide has an effect on the uptake of dieldrin by the microsomes (Table V).

The results confirm those of the first experiments.

Table V. Effect of cycloheximide on heptachlor epoxidase and microsomal dieldrin level in house flies treated with dieldrin.[1]

Treatment		Heptachlor Epoxide		Dieldrin in Microsomes
Dieldrin[2]	Cycloheximide[3]	μμM/fly	% of colony	ng/fly
Untreated	Untreated	70	100	0
Untreated	50 μg, homogenate	89	127	0
50 μg	50 μg, homogenate	235	336	34
50 μg	Untreated	169	241	26
50 μg	1 μg/fly, injected	63	90	22
100 μg	Untreated	211	301	42
100 μg	1 μg/fly, injected	88	126	39
200 μg	Untreated	177	253	60
200 μg	1 μg/fly, injected	72	103	69

[1] 100 flies/jar, three day old Orlando males.

[2] μg dieldrin per jar.

[3] Injected prior to dieldrin exposure, or added to homogenate.

Cycloheximide appears to cause an increase in epoxidase activity when added to the homogenate of dieldrin-treated flies (Table V). The reason for this apparent enhancement of the dieldrin effect is not known. It can be seen that cycloheximide has little effect on the dieldrin level in the microsomes.

These three experiments show that the change in enzyme activity brought on by dieldrin was completely stopped by the injection of the protein synthesis inhibitor. This suggests that dieldrin increases enzyme activity by an increase in protein synthesis.

A second method of determining whether dieldrin acted by stimulating the synthesis of protein was to measure the uptake of a C^{14} labeled amino acid in dieldrin treated house flies. Two different experiments were performed, one using 6 and one 10 day old Orlando males.

In the first experiment, 0.01 μC of UL-C^{14}-L-isoleucine was injected into each of 90 flies, then they were divided into three groups of 30. One group was topically treated with 0.5 μg dieldrin/fly, one group was treated with 1 μg cycloheximide/fly (cycloheximide was mixed and injected with the C^{14}-isoleucine), and one group received no further treatment. All the flies were held in small cages for 24 hours prior to homogenization. The three homogenates were processed as usual and the microsomal and soluble fractions were treated with TCA and counted, as explained in methods.

The results (experiment 1, Table VI) are shown as averages of duplicate C^{14} assays of protein fractions. The dieldrin treated flies incorporated approximately 10% more C^{14}-isoleucine than the controls, while the cycloheximide treated flies incorporated only one-fourth as much radioactivity.

In a second experiment only 15 flies were homogenized per sample. The amount of C^{14}-isoleucine injected was increased to 0.02 μC/fly and the dieldrin increased to 1 μg/fly. The cycloheximide treatment was replaced with a DDT treatment (1 μg/fly). The results (experiment 2, Table VI), indicate that the dieldrin treated flies incorporated approximately 3 to 6% more labeled amino acids into the microsomal and soluble fractions than did the colony flies. There was no difference in the mitchondrial fractions. The DDT treated flies incorporated less amino acids than the control flies.

The results of these experiments were not as conclusive as was hoped, possibly because of the time elapsing between the amino acid and dieldrin treatments and the preparation of the protein fractions. However, the striking effect of cycloheximide on protein synthesis confirms the results obtained in earlier experiments, Tables IV and V. Also, the depressing effect of water (Table IV) is seen to be unrelated to protein synthesis as all flies received labeled isoleucine as an aqueous solution.

The results of the cycloheximide and C^{14}-isoleucine

Table VI. Effect of treatment with dieldrin on amino acid incorporation into subcellular protein.

Treatment		dpm/mg protein		
L-Isoleucine	Insecticide	Mitochondria	Microsomes	Soluble
EXPT. 1[1]				
0.01 μC/fly	Untreated	--	2266	2890
0.01 μC/fly	0.5 μg Dieldrin/fly	--	2489	3210
0.01 μC/fly	0.5 μg Dieldrin + 1 μg Cycloheximide/fly[2]	--	386	940
EXPT. 2[3]				
0.02 μC/fly	Untreated	2987	4795	7498
0.02 μC/fly	1.0 μg Dieldrin/fly	2988	4949	7883
0.02 μC/fly	1.0 μg DDT/fly	2456	4803	7119

[1] 30, six day old Orlando males, average of two determinations with mean differences of less than 50 dpm.

[2] Cycloheximide injected with the isoleucine.

[3] 15, ten day old Orlando males, average of three determinations with mean difference of less than 30 dpm.

experiments provide evidence that dieldrin causes increased protein synthesis, and is therefore an inducing agent.

Characterization of the Inductive Effect

A second series of experiments was conducted to learn more about the interaction between dieldrin (and two other chlorinated hydrocarbons) and the protein synthesizing systems of the house fly. Naphthalene hydroxylation and heptachlor epoxidation were measured, as an indication of the response of the flies to the insecticides. Several measurements of naphthalene hydroxylation by house fly microsomes gave an average for control flies (Orlando) of 12.5 μμmoles product/fly/min with a mean difference of 0.84, and for dieldrin treated flies with an average of 36.8 with a mean difference of 0.89 μμmoles product/fly/min. Similar data for heptachlor epoxidase were given earlier.

Response to Inducing Agents

The first experiment involved treating two day old Orlando flies with aldrin or dieldrin and measuring the naphthalene hydroxylation activity after 24 and 48 hours. The results (Table I) show significant increases in activity in both groups of treated flies. Carbaryl mortality tests were also performed, and indicated that both insecticides cause decreases in mortality (Table VII).

Table VII. Mortality due to carbaryl in Orlando house flies exposed to aldrin or dieldrin for 24 and 48 hours.

Insecticide Treatment	Percent Mortality[1]			
	24 hours		48 hours	
	♀	♂	♀	♂
Untreated Colony	96	98	92	98
Aldrin, 75 μg/jar	--	44	--	64
Aldrin, 100 μg/jar	58	--	24	--
Dieldrin, 50 μg/jar	14	56	26	75

[1] 25 house flies/jar, 5 mg carbaryl/jar for males, 15 mg for females.

The activating effect of DDT was determined by treating Orlando and Fc females with topical doses for 3 and 24 hours. The Fc strain is unique among house fly strains studied by various workers because of its ability to oxidize DDT. It is apparent, from the results presented in Table VIII, that doses of DDT higher than 0.5 μg per fly depress microsomal epoxidase activity. Only at 0.1 μg is there evidence of stimulation of the Orlando flies. The data for the Fc strain, which shows stimulation at 0.1 and 0.25 μg, is in agreement with results reported by Gil et al.

These results (Table VIII) show that DDT is much less effective as an inducing compound than aldrin or dieldrin, the increase in activity being slight and greatly dependent on the dose. The inhibitory effect of high dose of DDT is also seen in the amino acid incorporation experiments (experiment 2, Table VI).

Table VIII. Heptachlor epoxidase activity of microsomes of Orlando and Fc house flies exposed topically to DDT for 3 and 24 hours.[1]

Insecticide Treatment	Heptachlor Epoxide Formed[2] μμmoles/fly			
	Orlando[3]		Fc[4]	
	3 hours	24 hours	3 hours	24 hours
Untreated colony	181	254	271	471
1 μg DDT	107	172	200	---
.5 μg DDT	136	174	219	---
.25 μg DDT	162	168	354	318
.1 μg DDT	212	228	287	382

[1] Flies topically treated with DDT and held in small cages for 3 or 24 hours.

[2] Incubation of 25 fly equivalents for 30 minutes.

[3] Orlando flies four days old.

[4] Fc flies ten days old.

Carbaryl Mortality of House Flies Pretreated with Dieldrin

Mortality tests were conducted with three house fly strains, dield;cyw, Orlando, and Isolan-R (males only) using carbaryl. Isolan-R females are immune to carbaryl and could not be investigated. Assays revealed that dieldrin treatments increased the tolerance to the toxicant in the Orlando and Isolan-R flies (examples of this effect are shown for Orlando flies in Tables IV, VII, IX, and X) but not the dield; cyw strain. In other mortality tests with the Orlando strain pretreated with dieldrin, there was no increase in

tolerance toMobam (4-benzothienyl-N-methylcarbamate) or naphthalene vapors. The results suggest that induction of detoxifying enzymes may protect the insect against other poisons only when the insect is already tolerant and the toxicant is readily detoxified, as is the case with carbaryl and the Isolan-R and Orlando strains.

Dosage Level and Length of Exposure

The relationship of dieldrin dose level and length of exposure to the response of the flies was studied in tests with one hundred three-day old Orlando males exposed to three levels of dieldrin (0.5, 5.0, and 50.0 μg per jar) for three periods (6, 12, and 24 hours). The tests were started so that all exposures were completed at the same time. Fifty flies from each group were used for enzyme assays and the other 50 for carbaryl mortality tests.

The results (Table IX) are expressed as percent enzyme activity compared to the activity of controls and as percent mortality to carbaryl. There is a dependence on both the dose and the duration of exposure. Five μg of dieldrin for 24 hours caused an increase in enzyme activity of 29% while 50 μg for 24 hours increased the enzyme activity by 108%. An appreciable decrease in mortality due to carbaryl was seen in the bioassays of the flies exposed to 50 μg dieldrin for 24 hours.

A second experiment compared the effect of five doses of

dieldrin on microsomal enzyme activities. Four day old female and male Orlando and dield; cyw flies were exposed to dieldrin residues of 5, 25, 50, 100, and 200 μg per jar for 24 hours. Microsomes were prepared from fifty flies and the microsomal pellet divided equally for assay of heptachlor epoxidase and naphthalene hydroxylase.

Table IX. Microsomal naphthalene hydroxylation and mortality due to carbaryl in Orlando male house flies pretreated with dieldrin.

Hours of exposure to dieldrin[1]	Naphthalene hydroxylation products, % of control[2]			% mortality after 24 hour exposure to carbaryl[3]		
	Dieldrin treatment, μg/jar					
	0.5	5	50	0.5	5	50
0				100	100	100
6	72	89	95	94	96	80
12	65	69	147	92	94	82
24	91	129	208	84	94	68

[1] Flies three days old at beginning of treatment.

[2] Untreated colony males.

[3] Twenty-five house flies and 5 mg carbaryl/jar, in duplicate.

From the results of this experiment (Figure 3), it can be seen that the activity of the two enzyme systems increased in parallel as the exposure to dieldrin increased, until a plateau was reached at the higher dosages.

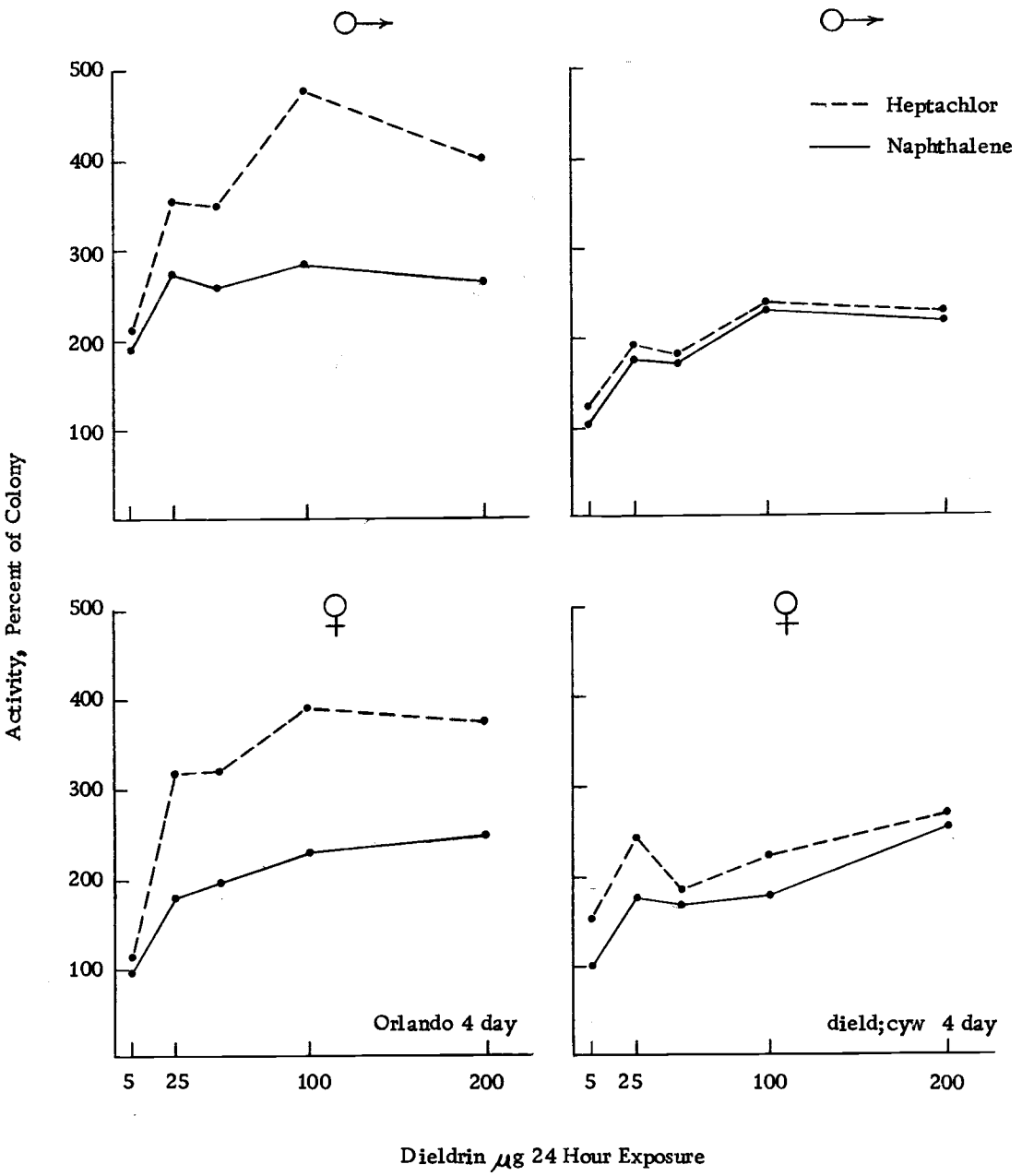


Figure 3. Relationship between level of dieldrin exposure and induction of microsomal oxidases in two dieldrin resistant house fly strains. Solid line, naphthalene hydroxylase; dashed line, heptachlor epoxidase.

Assays of the susceptibility to carbaryl of the Orlando strain indicated a pattern somewhat like the in vitro results, except for a reversal of the response at dosage levels above 50 μg (Table X). This may be due to the increasing levels of dieldrin in the fly tissues, as shown in Table XI.

Table X. Mortality due to carbaryl treatment in Orlando house flies pretreated with various levels of dieldrin.

Dieldrin Treatment[1]	Percent Mortality due to Carbaryl	
	Female[2]	Male[3]
Untreated colony flies	76	92
Dieldrin 5 μg/jar	87	82
Dieldrin 25 μg/jar	18	72
Dieldrin 50 μg/jar	12	42
Dieldrin 100 μg/jar	64	90
Dieldrin 200 μg/jar	72	96

[1] Four day old flies exposed in jars for 24 hours.

[2] Twenty-five flies and 15 mg carbaryl/jar, 24-hour exposure.

[3] Twenty-five flies and 5 mg carbaryl/jar, 24-hour exposure.

These experiments with various dosage levels of dieldrin indicate a definite relationship between dose and the magnitude of response.

Table XI. Level of dieldrin in microsomes prepared from house flies exposed to various levels of dieldrin.

Dieldrin Treatment[1]	µg Dieldrin per fly			
	Orlando		dield; cyw	
	♀	♂	♀	♂
Untreated colony flies	0	0	0	0
Dieldrin 5 µg/jar	2.7	2.5	1.8	2.2
Dieldrin 25 µg/jar	19.6	15.5	14.0	19.0
Dieldrin 50 µg/jar	36.4	42.5	29.0	33.0
Dieldrin 100 µg/jar	59.8	62.0	72.0	59.0
Dieldrin 200 µg/jar	119.0	117.0	95.0	103.0

[1] Four day old flies exposed in jars for 24 hours.

Induction: Relationship to Inherent Microsomal Oxidase Activity

A final group of experiments was conducted using the three dieldrin-resistant fly strains. A qualification of the results found by Schonbrod et al. was necessary when the level of epoxidase activity was surveyed over a period of 20 days. Schonbrod examined the activities for female flies at a single age, 12 days. The results in Table XII show that the level of epoxidase activity is dependent on the age of the flies. Young dield; cyw flies have more enzyme activity than Orlando flies the same age. Only after eight days are the Orlando flies more active than the dield; cyw flies. It was hypothesized that the oxidase activity of the strains might correlate with the

activating response to dieldrin.

Table XII. Effect of age on the level of microsomal epoxidase activity in house flies.[1]

	Heptachlor epoxide μμmoles/fly					
	dield; cyw		Orlando		Isolan-R	
Age	♀	♂	♀	♂	♀	♂
3	95.5	90.5	19.5	40.5	146.5	135.5
8	122.0	106.0	153.0	117.5	181.0	306.0
13	15.1	66.1	116.0	177.5	197.5	251.5
18	40.0	60.2	83.0	96.5	266.5	336.0
23	29.0	70.0	75.0	101.5	378.0	300.0

[1] Colony control values for figures shown in Figure 6.

Four day old females and five day old males of the three strains were given dieldrin treatments of 10, 50, and 150 μg per jar for 24 hours. Enzyme assays were done as usual. The data is expressed as change in enzyme activity, μμmoles/fly, obtained by subtracting the enzyme activity of the colony controls from that of the dieldrin treated flies.

The results (Figure 4) reveal a marked difference between the three strains. The Isolan-R females gave the greatest response of female flies tested, with the dield; cyw giving the least. The males show a leveling off in response to high dieldrin doses as has been previously noted (see Figure 3). However, the Isolan-R males

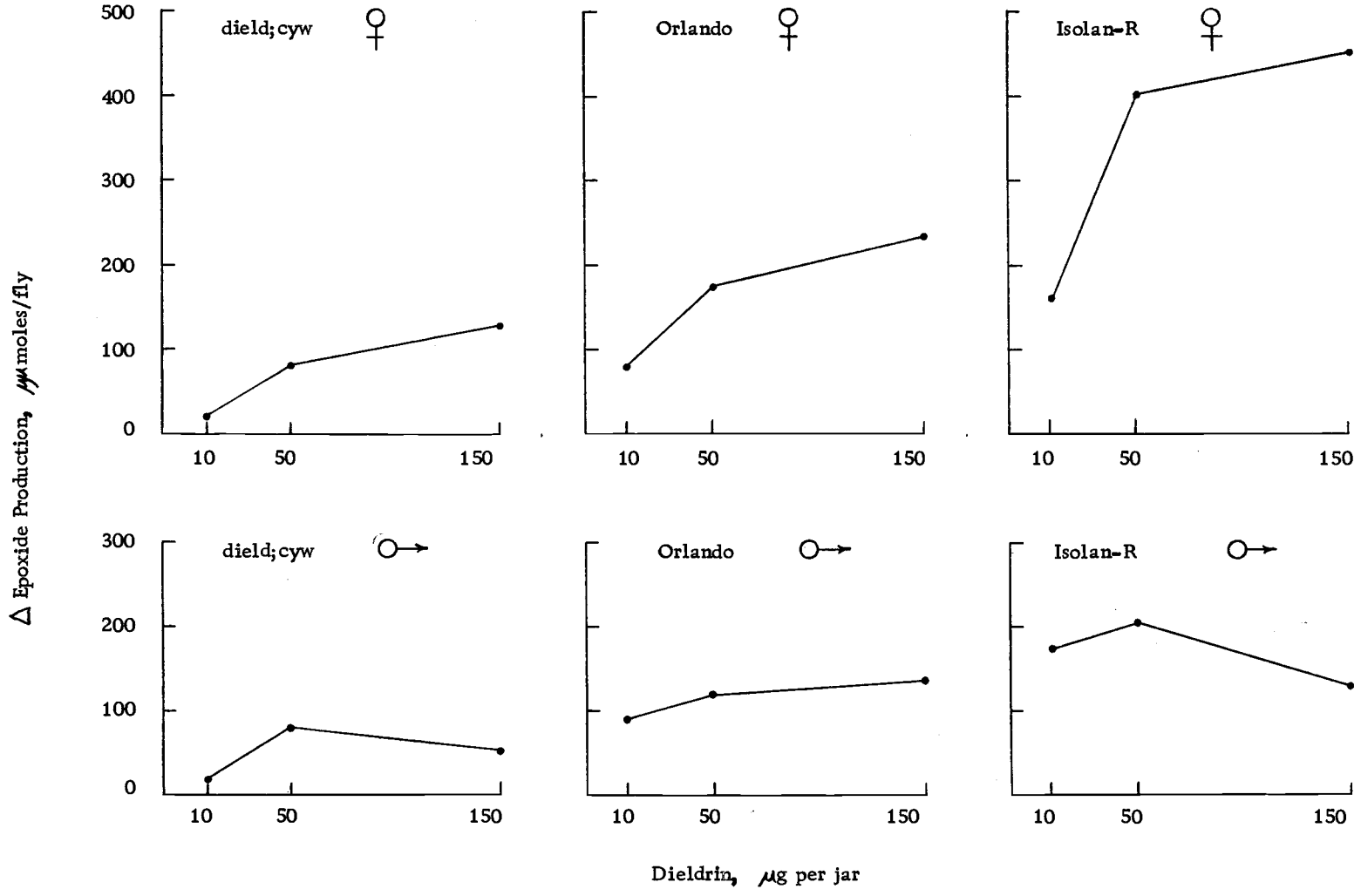


Figure 4. Increase in microsomal epoxidase activity as related to dose in three house fly strains. Four day old females, five day old males exposed to dieldrin at 50 μg per jar, 24 hours. Colony control values subtracted to give Δ epoxide production.

initially showed a greater response than either the Orlando or dield; cyw strains. The difference in dieldrin content of the microsomes was negligible indicating that uptake of the inducer was the same in the three strains.

Effect of Topical Treatment with Dieldrin

Dieldrin (0.5 μg) was applied topically and the effect of exposure time observed. Three day old males and females of the three strains were tested at 0, 3, 6, 12, 18, and 24 hours after treatment. The assays were performed as usual. All strains required a minimum of 12 hours for a significant response to the treatment. The strains differed in magnitude of the responses (Figure 5), but in the same order as in the previous experiment. The response was greatest for both male and female Isolan-R, with Orlando intermediate, and dield; cyw least. The dieldrin level in the microsomes was similar for all flies assayed. This experiment supports the previous findings indicating that strains with higher microsomal oxidase activities are more responsive to dieldrin treatment.

Duration of Inductive Effect

In addition to studying differences in strain response, this experiment was designed to determine the effect of age of the fly and of additional dieldrin treatment on microsomal oxidase activity.

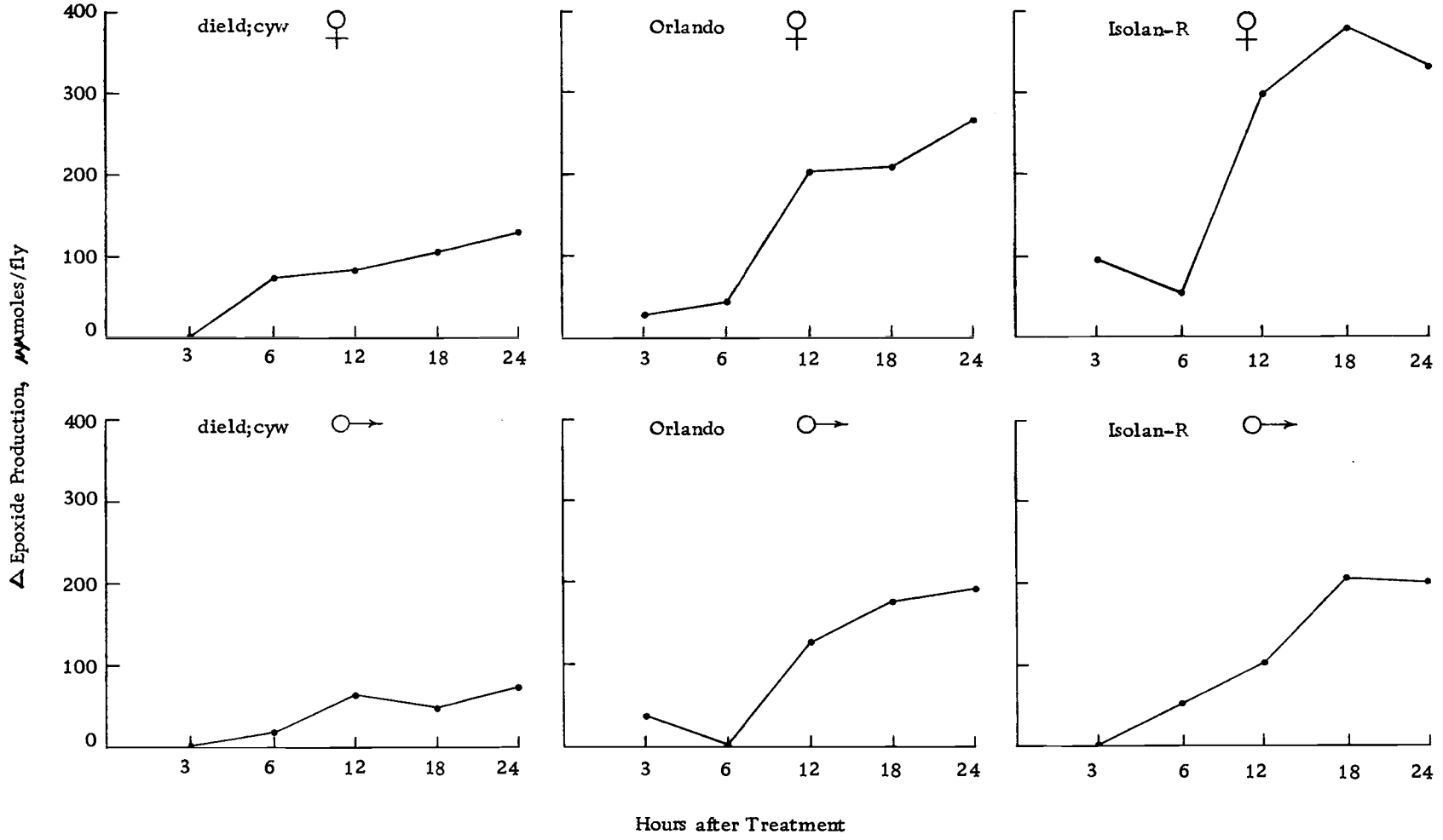


Figure 5. Increase in microsomal epoxidase activity as related to time after topical treatment in three house fly strains. Three day old flies treated with 0.5 μg of dieldrin per fly. Colony control values subtracted to give Δ epoxide production.

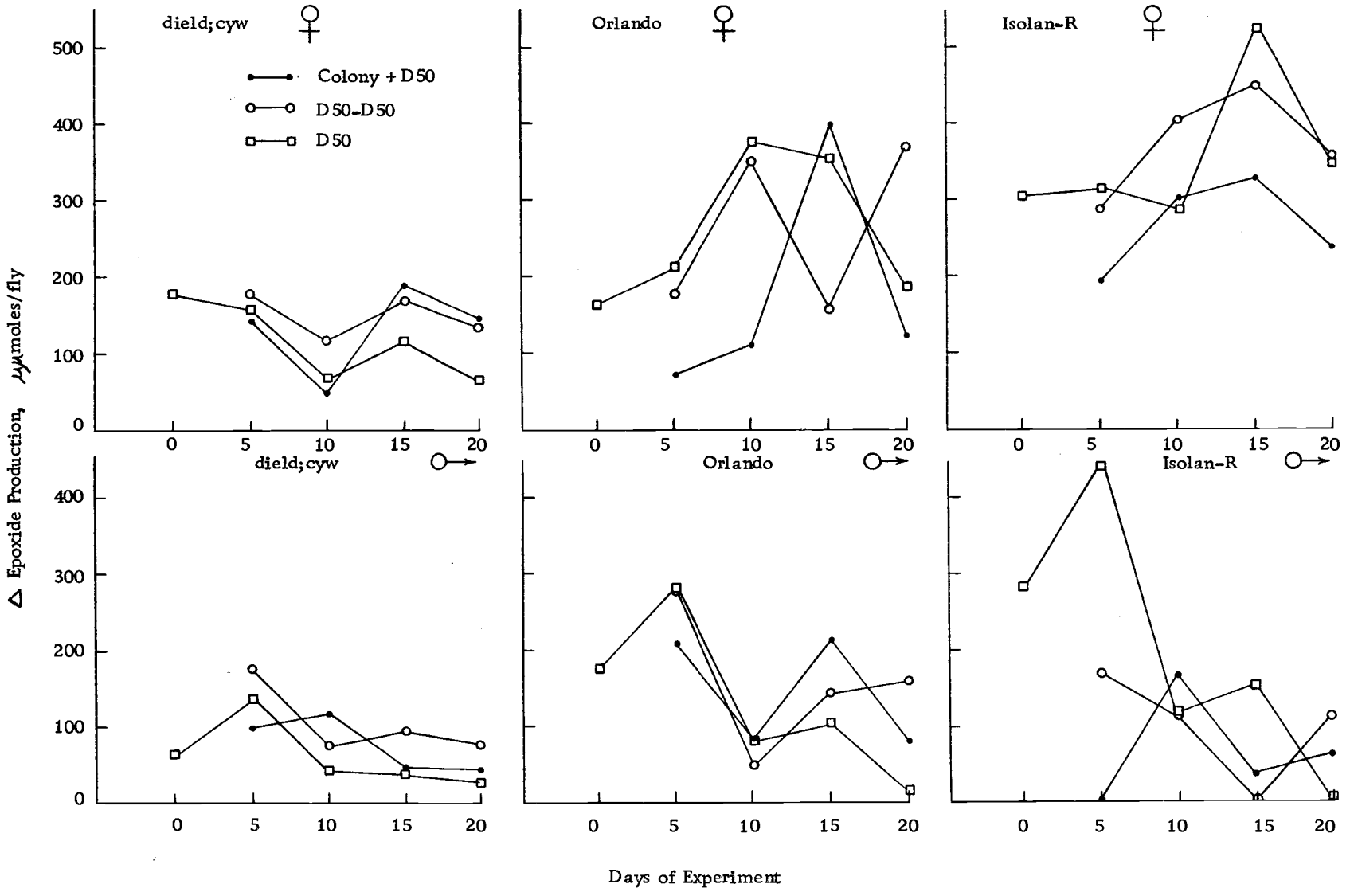
A large number of three day old flies of both sexes of the three strains were treated with 50 μg of dieldrin per jar. After 24 hours a sample was taken, microsomes were prepared, and enzyme activity was compared with that of colony flies. The remaining flies were placed in wire cages and samples were removed at five day intervals for enzyme activity measurements. Prior to each assay, another subgroup of flies was reexposed to the same dose of dieldrin. To determine the effect of age, a sample of colony flies was taken prior to microsome preparation and treated with 50 μg of dieldrin for 24 hours, and then assayed with the other groups. This procedure was followed for 20 days.

The results show (Figure 6) that the three strains responded differently to the dieldrin treatments. The enzyme activity after a single dieldrin treatment corresponded to the previous results (Figures 4 and 5) with the Isolan-R flies giving the greatest response, Orlando next, and dield; cyw the least. The age of the flies affected the magnitude of response, but not the potential to respond. The duration of response was prolonged, probably due to dieldrin storage in the fly microsomes. However, in the males treated with a single dose, microsomal dieldrin levels decreased with time, and the enzyme activity after 20 days was the same as colony flies. The response to retreatment was erratic. This may be due to the accumulation of dieldrin in the flies, which has been shown

Figure 6. Duration of inductive effect in three house fly strains. Treatments as follows:

1. Three day old flies exposed to dieldrin (50 μg/jar, 24 hours) were assayed for microsomal epoxide activity at five day intervals (D50).
2. Sub group of # 1 retreated one day prior to assay (D50 + D50).
3. Previously untreated, exposed to dieldrin one day prior to assay (Col + D50).

Colony control values subtracted to give Δ epoxide production.


dield;cyw
Orlando
Isolan-R
Colony + D50
D50-D50
D50
Δ Epoxide Production, μμmoles/fly
Days of Experiment

previously to reduce responses (Figure 3). Investigations of this factor have not been detailed enough to permit an understanding of the results, therefore the effect of retreatment is not clear.

In all treatments, the Isolan-R and Orlando flies exhibited more fluctuation in level of response than the dield; cyw flies (this may reflect heterozygosity in the strains). However, in all experiments, the response of the Isolan-R strain was the largest. This strongly suggests a relationship between the level of enzyme activity of a strain and its magnitude of response to dieldrin treatment.

GENERAL DISCUSSION

These experiments have established that aldrin or dieldrin treated house flies exhibit an increase in microsomal oxidase activity. Flies treated with cycloheximide before exposure to dieldrin showed no enzymatic response and C^{14}-isoleucine incorporation was greatly retarded. Dieldrin-treated flies, however, showed increased uptake of isoleucine. Kinetic studies further indicated that dieldrin treatment resulted in higher rates of oxidation rather than in changes in enzyme affinity for the substrate. From these studies, it was concluded that dieldrin caused an increase in protein synthesis and can be termed an inducing agent.

It was found that the magnitude of response to dieldrin, but not the potential to respond, was related to the level of oxidase activity of house fly microsomes. This conflicts with previous work with DDT-resistant Triatoma infestans nymphs and DDT-resistant house flies, which has indicated that a high level of oxidase activity is a prerequisite for induction. The effect of dieldrin on the dield; cyw strain, which has a low microsomal oxidase activity, indicates that tolerance to the inducing compound is a prerequisite for induction only because it permits the use of toxic inducers.

The characterization of the effect of dieldrin revealed that the magnitude of response depends on the dose and on the duration of the

treatment. Flies were found to be inducible at all ages, with a reduction in magnitude observed in older flies. The return to normal enzyme activity is slow probably because of storage of dieldrin in the flies. Although the results were erratic, retreatment did not produce an appreciable increase in response over singly treated flies.

The classification of the three strains by levels of oxidase activity was complicated by the effect of age. Based on the maximum value of oxidase activity, the three strains can be ranked according to their potential oxidase activity level. On the basis of such a classification, it was found that the strain with the highest potential showed the greatest response to dieldrin treatment. The order was Isolan-R, Orlando and dield; cyw.

The findings of Gil et al. (1968) using DDT to induce microsomal enzymes in house flies, were substantiated using one of the strains they investigated, Fc. However, tests using the Orlando strain were negative. This may be related to the fact that DDT is metabolized by microsomal enzymes in the Fc strain, and by soluble enzymes in the Orlando strain. Alternatively, the Fc strain (as with the Isolan-R strain and dieldrin) may be more induceable by DDT than the Orlando strain. In any event, the studies with DDT indicate the dependence of dose on the response observed. Dieldrin exhibited a wider range of dose effect level.

The synthesis of some proteins in microorganisms and higher

animals is constitutive, that is, such proteins are synthesized at a constant rate and are unaffected by changes in cellular environment. Synthesis of other proteins can adjust to changes in cellular environment and is thus inducible. The mechanism of induction of microsomal oxidases in house flies is not known but may be similar to one proposed for microorganisms (Jacob and Monod, 1961). They propose a mechanism where in the synthesis of certain proteins is regulated by means of repressor proteins binding to specific areas on the bacterial chromosome. This idea has been supported by Gilbert (1966 and 1967), Ptashne (1967a and 1967b), Ptashne and Hopkins (1968) and Riggs et al. (1968) who have isolated repressor proteins and have shown that they bind to DNA on specific regions of the chromosome. Other mechanisms provide for regulation at other steps in protein synthesis.

Two explanations for the origins of different levels of microsomal oxidases in house fly strains are suggested by the results of this study. The Isolan-R strain shows the greatest response to dieldrin thus may have more genes governing synthesis of oxidases, conversely, this strain may have the same number of genes as the others with a different degree of regulation. It follows from these studies, that dieldrin interacts with a regulatory mechanism and causes an increase in protein synthesis. Whether this interaction is between several regulatory sites or a single one can not be

ascertained from this work. Either explanation accounts for genetic studies in insects that show that the genes responsible for insecticide detoxicating enzymes are inherited as dominant and semi-dominant factors (Oppenoorth, 1959; Franco and Oppenoorth, 1962; Hoyer, Plapp and Orchard, 1965; Plapp and Hoyer, 1967).

In summary, this investigation has shown that aldrin and dieldrin can increase microsomal oxidase activities in house flies. It is concluded that this effect is due to induction of protein synthesis and that the magnitude of response to dieldrin is related to the level of microsomal oxidase.

BIBLIOGRAPHY

Agosin, M., D. Michaeli, Miskus, R., S. Nagasawa and W. M. Hoskins. 1961. A new DDT-metabolizing enzyme in the German cockroach. Journal of Economic Entomology 54:340-342.

Agosin, M. and M. L. Dinamarca. 1963. The effect of DDT on the level of di- and triphosphopyridine nucleotides in Triatoma infestans. Experimental Parasitology 13: 199-203.

Agosin, M., N. Scaramelli, M. L. Dinamarca and L. Aravena. 1963. Intermediary carbohydrate metabolism in Triatoma infestans nymphs and the effect of DDT. Comparative Biochemistry and Physiology 8: 311-320.

Agosin, M., L. Aravena and A. Neghine. 1965. Enhanced protein synthesis in Triatoma infestans treated with DDT. Experimental Parasitology 16: 318-324.

Agosin, M., B. C. Fine, N. Scaramelli, J. Ilivicky and L. Aravena. 1966. The effect of DDT on the glucose and glycine into various intermediates in DDT-resistant strains of Musca domestica L. Comparative Biochemistry and Physiology 19: 339-349.

Agosin, M., J. Ilivicky and S. Litvak. 1967. The induction of NAD Kinase by DDT in Triatoma infestans. Canadian Journal of Biochemistry 45: 619-626.

Balazs, I. and M. Agosin. 1968. The effect of 1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl)ethane on ribonuclecic acid metabolism in Musca domestica L. Biochemica et Biophysica Acta 157: 1-7.

Chakraborty, J. and J. N. Smith. 1967. Enzymatic oxidation of some alkylbenzenes in insects and vertebrates. Journal of Biochemistry 102: 498-503.

Dixon, M. and E. C. Webb. 1964. Enzymes. 2d ed. New York, Academic. 950p.

Ennis, H. L. and M. Lubin. 1964. Cycloheximide: Aspect of inhibition of protein synthesis in mammalian cells. Science 146: 1474-1476.

Franco, M. and F. J. Oppenoorth. 1962. Genetical experiments on the gene for low aliesterase activity and organophosphate resistance in Musca domestica L. Entomologia Experimentalis et Applicata 8: 65-73.

Gil, L., B. C. Fine, M. L. Dinamarca, I. Balazs, J. R. Busvine and M. Agosin. 1968. Biochemical studies on insecticide resistance in Musca domestica. Entomologica Experimentalis et Applicata 11: 15-29.

Gilbert, W. and B. Muller-Hill. 1966. Isolation of the lac repressor. Proceedings of the National Academy of Sciences 56: 1891-1898.

_____ 1969. The lac operator is DNA. Proceedings of the National Academy of Sciences 58: 2415-2421.

Hoyer, R. F., F. W. Flapp, Jr. and R. D. Orchard. 1965. Linkage relationships of several insecticide resistance factors in the house fly. Entomologia Experimentalis et Applicata 8: 65-73.

Ilivicky, J., M. L. Dinamarca and M. Agosin. 1964. Activity of NAD-Kinase of nymph Triatoma infestans upon treatment with DDT and other compounds. Comparative Biochemistry and Physiology 11: 291-301.

Ilivicky, J. and M. Agosin. 1967. Glutathione turnover in Triatoma infestans treated with 2, 2-bis-(p-chlorophenyl)-1, 1, 1-trichloroethane. Experimental Parasitology 20: 345-356.

Jacob, F. and J. Monod. 1961. Genetic regulatory mechanisms in synthesis of proteins. Journal of Molecular Biology 3: 318-365.

Litvak, S., L. Tarrago-Litvak, P. Poblete and M. Agosin. 1968a. Evidence for the DDT-induced synthesis of messenger ribonucleic acid in Triatoma infestans. Comparative Biochemistry and Physiology 26: 45-56.

Litvak, S. and M. Agosin. 1968b. Protein synthesis in polysomes from house-flies and the effect of 2, 2-bis (p-chloro-phenyl)-1, 1, 1-trichloroethane. Biochemistry 7: 1560-1567.

Meksongsee, B., R. S. Yang and F. E. Guthrie. 1967. Effect of inhibitors and inducers of microsomal enzymes on the toxicity of carbamate insecticides to mice and insects. Journal of Economic Entomology 60: 1469-1471.

Morello, A. 1964. Role of DDT-hydroxylation in resistance. Nature 203: 785-786.

Oppenoorth, F. J. 1959. Genetics of resistance to organophosphorous compounds and low aliesterase activity in the house fly. Entomologia Experimentalis et Applicata 2: 304-319.

Oppenoorth, F. J. 1967. Two types of sesamex-suppressible resistance in the house fly. Entomologia Experimentalis et Applicata 10: 75-86.

Oppenoorth, F. J. and N. W. H. Houx. 1968. DDT-resistance in the house fly caused by microsomal degradation. Entomologia Experimentalis et Applicata 11: 81-93.

Plapp, F. W., Jr. and R. F. Hoyer. 1967. Insecticide resistance in the house fly: Resistance spectra and preliminary genetics of resistance in eight strains. Journal of Economic Entomology 60: 768-774.

Ptashne, M. 1967a. Isolation of the λ repressor. Proceedings of the National Academy of Sciences 57: 306-313.

_____ 1967b. Specific binding of the λ phage repressor to the DNA. Nature 214: 232-234.

Ptashne, M. and N. Hopkins. 1968. The operators controlled by the λ phage repressor. Proceedings of the National Academy of Sciences 60: 1282-1287.

Riggs, A. D., S. Bourgeois, R. F. Newby and M. Cohn. 1968. DNA binding of the lac repressor. Journal of Molecular Biology 34: 365-368.

Schafer, J. A. 1969. Naphthalene resistance in a house fly strain: Its inheritance and characteristics. Master's thesis. Corvallis, Oregon State University. 47 numb. leaves.

Schonbrod, R. D., W. W. Philleo and L. C. Terriere. 1965. Hydroxylation as a factor in resistance in house flies and

blow flies. Journal of Economic Entomology 58: 74-77.

Schonbrod, R. D., M. A. Q. Khan, L. C. Terriere and F. W. Plapp, Jr. 1968. Microsomal oxidases in the house fly: A survey of fourteen strains. Life Sciences 7: 681-688.

Schonbrod, R. D. 1969. Assistant in Entomology, Oregon State University, Dept. of Entomology. Personal communication. Corvallis, Oregon.

Street, J. C. 1968. Modification of animal responses to toxicants. In: The enzymatic oxidation of toxicants: Proceedings of a conference at North Carolina State University, Raleigh, 1968. Raleigh, North Carolina State University. 228p.

Tsukamoto, M. and J. E. Casida. 1967. Albumin enhancement of oxidative metabolism of methyl carbamate insecticide chemicals by the house fly microsome-$NADPH_2$ system. Journal of Economic Entomology 60: 617-619.