EFFECTS OF X-IRRADIATION OF THE HOUSE FLY ON CHOLINESTERASE ACTIVITY

by

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ABSTRACT

Colorimetric and radiometric assays were used to measure the level of *in vitro* cholinesterase (ChE) activity in non-irradiated house flies and those X-irradiated as pupae with 5,000, 10,000, or 15,000 R. No difference in ChE activity was found between the two groups whether the ChE source was males or females, fly heads, headless flies or whole flies.

A radiometric assay was used to measure the *in vitro* susceptibility of ChE to inhibition by malathion or malaoxon in flies X-irradiated as pupae with 10,000 R and non-irradiated flies. There was no difference between the two groups in ChE inhibition by malathion or malaoxon in males, females, fly heads, thoraces or headless flies.

The possibility is discussed that inhibition of ChE or altered ChE activity in a critical area of the nervous system could have been masked in these assays. The possibility is also discussed that decreased malathion toxicity in flies from irradiated pupae may be attributed to inhibition of desulfurase by X-radiation.

INTRODUCTION

An altered susceptibility of insects to insecticides following irradiation has been demonstrated in several studies; however, the mechanism of this phenomenon has not been determined. The mechanism could involve alterations in penetration of the insecticide to the target organ, metabolism of the insecticide, or activity of the target. The role of the target enzyme, cholinesterase (ChE), in the altered susceptibility of the house fly, Musca domestica L., to malathion and malaoxon following X-irradiation of the pupae was investigated in this thesis.

The effects of radiation on the susceptibility of house flies to malathion have been extensively investigated. Guenthner and Ware (1967) reported that X-irradiation of house fly pupae reduced the toxicity of malathion to adult females but produced no effect on adult males. When house flies were irradiated as adults, no change in susceptibility to malathion was observed in males or females. However, Whitacre and Ware (1970) reported a reduced susceptibility of both adult male and female house flies to malathion following either gamma- or X-irradiation of pupae.

The combined effects of piperonyl butoxide and malathion or malaoxon applied topically to adult house flies X-irradiated as pupae were investigated by Ware and Whitacre (1970). Malathion was less toxic to irradiated flies than to non-irradiated flies. Piperonyl

butoxide applied topically with malathion exaggerated this difference in toxicity and also decreased the toxicity to both irradiated and non-irradiated flies. Malaoxon was more toxic to irradiated flies than to non-irradiated flies. Piperonyl butoxide applied topically with malaoxon reduced this difference in toxicity, but it increased the toxicity to both irradiated and non-irradiated flies.

The susceptibility of irradiated insects to azinphosmethyl has also been investigated. Rush and Ware (1969) observed an increased toxicity of azinphosmethyl to adults of the pink bollworm, *Pectinophora gossypiella* (Saunders), following gamma-irradiation of the pupae. However, Wolfenbarger and Graham (1970) reported no difference in susceptibility of pink bollworm moths to this compound when irradiated as adults. The author found no difference in toxicity of azinphosmethyl to adult house flies following X-irradiation of the pupae with 6250 R.

The toxicity of several other insecticides has been found to be altered by irradiation of the insect. Preliminary studies of 35 insecticides by John L. Drake (Department of Entomology, University of Arizona) showed that the toxicity of 6 organophosphate insecticides to adult house flies were significantly different at the 95% level following X-irradiation of the pupae. The most pronounced differences included the decreased toxicity of Abate® to females with no effect on males, and the increased toxicity of Meta-Systox-R® to males and females. Varzandeh and Moos (1963) showed that adult house flies from pupae receiving high dosages of X-radiation were more susceptible to DDT than those receiving low dosages. Hough (1963) found a decreased

toxicity of DDT to adults of the codling moth, *Carpocapsa pomonella* (L.), which has been gamma-irradiated as eggs less than one day old. Temik® was found to increase in toxicity to irradiated male and female house flies, but it decreased in toxicity to male house flies irradiated as pupae with no effect on females (Guenthner and Ware, 1967). Vashkov and Poleshchuk (1966) reported that immature female house flies became more susceptible to DDT, benzene hexachloride, and trichlorphon following X-irradiation. Radiation-sterilized oriental fruit flies, *Dacus dorsalis* Hendel; Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann); and the melon fly *D. cucurbitae* Coquillett were less susceptible to DDT and malathion that non-irradiated flies (Keiser and Schneider, 1969).

Of the insecticides which have been reported to have radiation-induced changes in toxicity, malathion appears to be the most distinctly and consistently influenced by radiation in several species and under varied conditions (Table 1). Therefore, malathion and its oxygen analogue, malaoxon were the insecticides of choice in this investigation.

The metabolism and mode of action of malathion have been extensively investigated (O'Brien, 1967). In mammals and insects malathion is activated by a desulfurase which converts malathion to the highly toxic molecule, malaoxon. Malathion is detoxified in mammals and insects by a phosphatase, which cleaves the phosphate ester linkages in organophosphate insecticides. It is also cleaved by carboxyesterase which breaks the carboxyl ester linkage, characteristic of

Table 1. The effect of irradiation of pupae on malathion and malaoxon toxicity to adult insects.

Insecticide, Insect	Radiation Level	Average Mortality			
		Males		Females	
		Control	Irrad.	Control	Irrad.
Guenthner and Ware (1967)					
Malathion		Percent Mortality			
Musca domestica	7,500 R	56.6	55.6	72.3	39.3
	10,000 R	56.6	47.5	72.3	37.6
Whitacre and Ware (1970)					
Malathion					
Musca domestica		LD_{50} Values			
24 hour pupae	5,000 R	18.2	26.2	22.2	26.2
72 hour pupae	5,000 R	27.3	29.0	29.3	38.3
Ware and Whitacre (1970)					
Musca domestica		LD_{50} Values			
Malathion	10,000 R	19.2	23.4	18.4	29.6
Malathion + PB[a]	10,000 R	32.4	24.3	58.5	60.3
Malaoxon	10,000 R	23.3	21.1	23.7	17.8
Malaoxon + PB[a]	10,000 R	9.2	7.1	8.2	8.0
Keiser and Schneider (1969)					
Malathion		LD_{50} Index[b]			
Dacus dorsalis	10,000 R	1.71			
Dacus cucurbitae	10,000 R	1.70			
Ceratitis capitata	10,000 R	2.23			

a) piperonyl butoxide

b) Indices represent the number of times more insecticide required to achieve LD_{50} level for irradiated flies compared with that for non-irradiated flies.

the alkyl side chains in malathion and a few other organophosphate insecticides. Malathion is attacked by ChE which then becomes inhibited by the attached phosphate group of the malathion. This leads to the death of the animal when cholinesterase is inhibited in vital synapses (Molloy, 1961; Booth and Metcalf, 1970).

The activity of any of the above enzymes could be affected by whole-body irradiation, and each of them has been shown to be involved in selective resistance or susceptibility of various species to organophosphate insecticides (O'Brien, 1967). It seems likely, however, that a combination of these enzymes and possibly physical factors are involved in the mechanism of the alteration of malathion toxicity by radiation.

The present investigation involved two major comparisons between non-irradiated flies and those X-irradiated as pupae: (1) a comparison of the levels of total *in vitro* ChE activity, and (2) a comparison of the *in vitro* susceptibility of ChE to inhibition by malathion or malaoxon. ChE was chosen to be studied because of its importance as the target enzyme for malathion. These results should help explain the mechanism of the radiation-induced changes in the toxicity of organophosphate insecticides.

MATERIALS AND METHODS

Rearing and Irradiation Methods

A Chemical Specialities Manufacturer's Association (CSMA) susceptible strain of house fly was used throughout this project. Larvae were reared in CSMA standard dry larval medium, following the Peet-Grady Method (Anonymous, 1961) at a temperature of 25±2°C and a relative humidity of 20-50%. After separation from the larval medium, 20.0 ml of pupae were randomly divided into a 10.0 ml test group and a 10.0 ml control group.

The test group was irradiated 2 days before peak emergence, the time at which irradiation of pupae produces adults least susceptible to malathion (Whitacre and Ware, 1970). The day of irradiation came 2 or 3 days after pupation was initiated, depending on the length of the pupal stage. The test pupae received 1,250 R per minute from a General Electric Maximar 400 therapy X-ray unit modified for irradiation of insects (Ware and Irwin, 1969). The container of control pupae in the radiometric assays was placed adjacent to the test pupae but was shielded during irradiation by a ¼-inch thick lead strip, which was bent to permit air circulation among the pupae. In the colorimetric assays the control pupae were rotated on the irradiation platform for 5 minutes but received no X-radiation.

The test and control pupae were then placed in separate manila quart ice cream cartons with mosquito netting lids and were maintained

at 27±2°C and 40-60% relative humidity with a photoperiod of 14 hours of light and 10 hours of dark. Two sugar cubes were placed in each carton. Cotton wads, soaked in water and protected from evaporation by aluminum foil, were placed on the mosquito netting lids and replaced daily. Both groups of flies were assayed approximately 72 hours after peak emergence, the time at which Whitacre and Ware (1970) conducted their malathion toxicity studies.

Colorimetric Assay for Cholinesterase Activity

A colorimetric assay was used to determine the level of total ChE activity in X-irradiated and non-irradiated flies. The procedures of Hestrin (1949) were followed with little modification except that the volumes of the solutions were altered for use with fly homogenates. The following amounts of flies were weighed and then homogenized in 5.0 ml cold phosphate buffer, pH 7.2, in a glass homogenizer: 4 male or 3 female whole bodies, 8 male or 8 female heads, or 8 male or 7 female headless flies. The homogenate was centrifuged 5 minutes at 10,000 rpm, and 1.0 ml of the supernatant was mixed with 1.0 ml 4mM acetylcholine chloride (AChCl) in 1mM sodium acetate. The solution was incubated in a Warburg bath at 37°C for 20 minutes. The reaction was stopped by adding 2.0 ml of a premixed solution of 2M hydroxylamine and 3.5N sodium hydroxide (1:1, v/v), which formed a complex with the unhydrolyzed AChCl. Then enough 4M hydrochloric acid (HCl) was added to bring the pH to 1.2±0.2. The mixture was centrifuged 5 minutes at 10,000 rpm to remove any suspended precipitate. Then 1.0 ml 0.37M ferric chloride was added to color the acetylcholine complex brown.

The solution was immediately transferred to a quartz cuvette and the bubbles adhering to the sides were removed with a fine brush. Reference blanks used to correct for non-enzymatic hydrolysis were prepared similarly except that alkaline hydroxylamine and HCl were added in reverse order. The solutions were read immediately at 535 nm on a Hitachi Perkin-Elmer spectrophotometer, model 139. For each sample, the μM AChCl hydrolyzed per mg fly per hour was calculated.

A standard curve of AChCl concentration vs. optical density (Figure 1) was plotted with 4 concentrations of AChCl and a reference blank for each replicate, and all of which were prepared as above except that phosphate buffer was substituted for homogenate. The homogenate concentration used in the assays was in a linear portion of the curve representing homogenate concentration vs. ChE activity under the conditions described above (Figure 2).

Radiometric Assay for Cholinesterase Activity

The Gaballah (1968) radiometric assay for ChE was modified for use with house fly homogenates. One fly, male or female, irradiated or non-irradiated was weighed and then homogenized in 1.0 ml cold phosphate buffer, pH 7.2, in a glass homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 minutes, and 50 μl of the supernatant was mixed with 50 μl of 4mM (acetyl-1-^{14}C)choline chloride (^{14}C-AChCl)[1] with an activity of 0.2 μCi/50 μl. This solution was incubated in a

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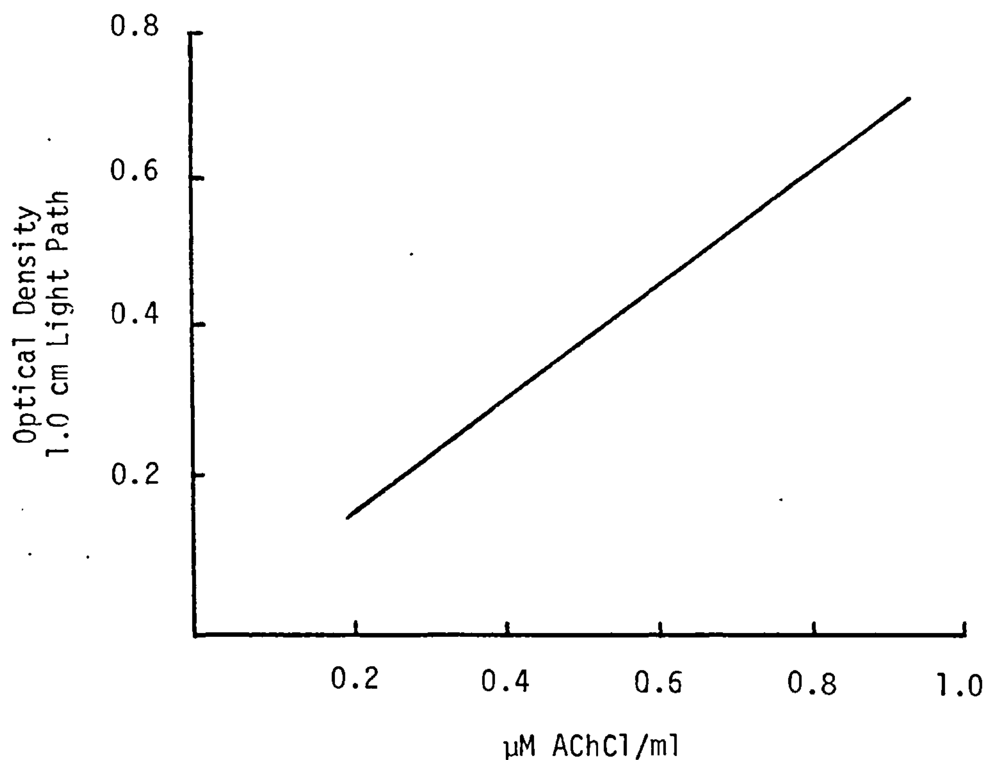
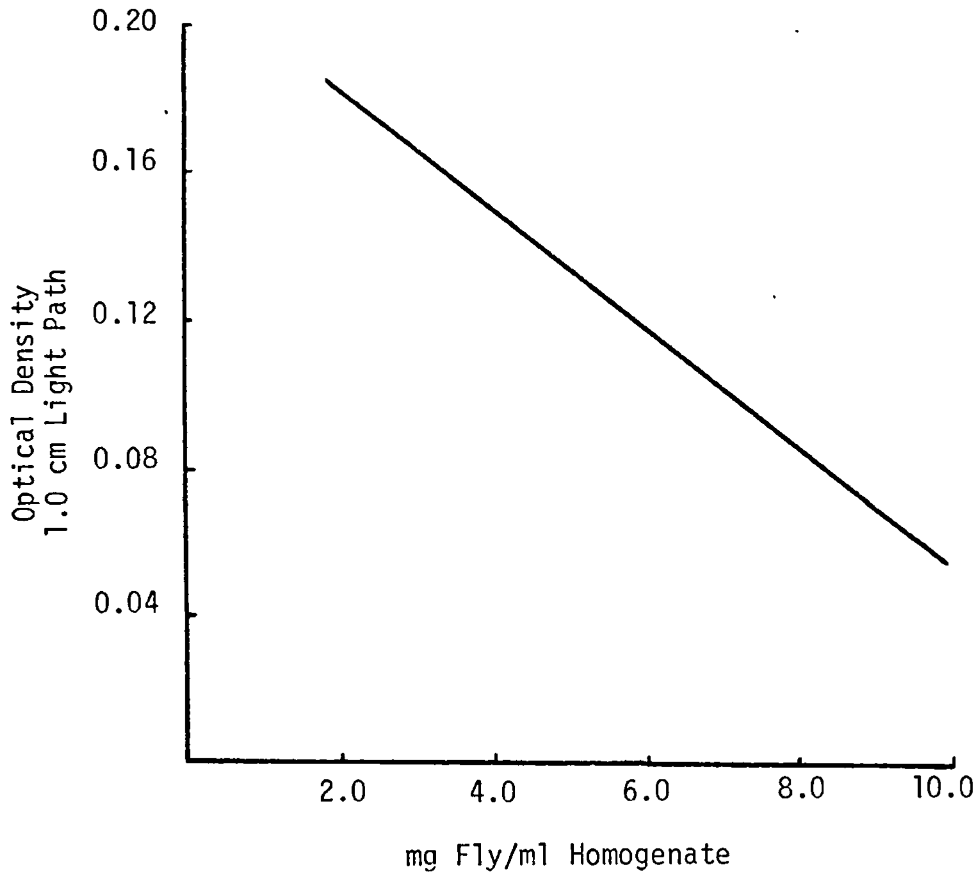
1. ^{14}C-AChCl (9.2 mCi/mM) was obtained from Amersham/Searle Corp., Des Plaines, Illinois.



r = 0.9821
Significant at 1% Level
y = 0.7452x + 0.0058

Figure 1. Standard curve for acetylcholine chloride (AChCl) concentration, representing the combined points of all replicates of the colorimetric assay.



r = -0.8704
Significant at 1% Level
y = -0.0160x + 0.2110

Figure 2. Cholinesterase (ChE) activity in male flies as a function of initial homogenate concentration in the colorimetric assays.

Acetylcholine chloride was present at a final concentration of 0.4 μM/ml. Optical density is inversely proportional to ChE activity.

Warburg bath at 37°C for 5 minutes. The solution was then chilled on ice to prevent non-enzymatic hydrolysis of ^{14}C-AChCl and suppress ChE activity. Then 100 µl 0.2N HCl was immediately added to suppress ionization of enzymatically liberated radioactive acetic acid. The free acetic acid was extracted by shaking the solution with 2.0 ml toluene and isoamyl alcohol (9:2, v/v). The mixture was centrifuged at 10,000 rpm for 5 minutes, and a 1.0 ml aliquot of the toluene phase was added to 15 ml fluor in a glass counting vial with a foil-lined cap. The fluor was made by dissolving 0.06 g PPO and 5.00 g POPOP[2] in 1.0 liter reagent grade toluene. A blank used to correct for non-enzymatic hydrolysis was prepared as above except that the homogenate was boiled for 10 minutes before incubation with ^{14}C-AChCl.

The samples were counted in a Nuclear Chicago ambient temperature liquid scintillation counter, Model 6822, at an efficiency of approximately 50%. The total counts per sample were made large enough that the confidence level for counts per minute was above 90%. A standard quench curve, obtained by the channels ratio method, was used to determine percent efficiency. Seventy-five microliters (1.9 µCi) of aqueous ^{14}C-AChCl was slowly mixed first with 0.5 ml ethanol and then with 1.5 ml isoamyl alcohol. Ten milliliters of toluene were then slowly added to the solution. A 1.0 ml aliquot was slowly mixed with 15.0 ml fluor to make a final radioactivity of 0.158 µCi per counting vial. Seven vials received 0, 10, 20, 30, 40, 50, and 60 µl

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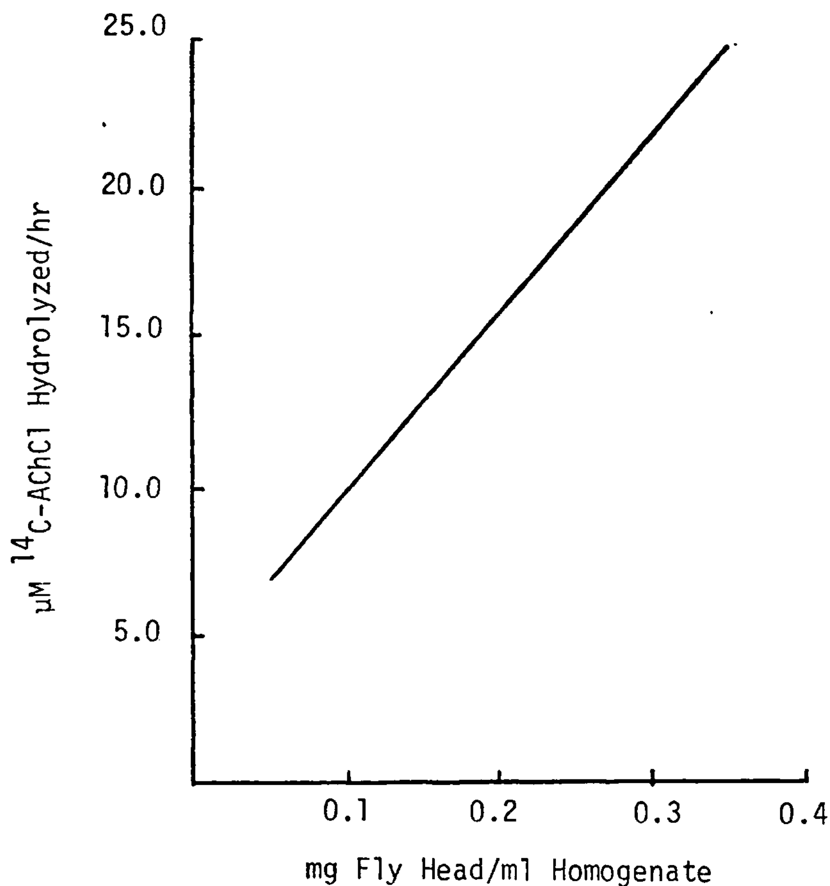
2. PPO (2,5-diphenyloxazole) and POPOP (p-bis-[2-(5 phenyloxazolyl)]-benzene) were obtained from Nuclear Chicago Corp., Des Plaines, Illinois.

respectively of nitromethane, a very effective chemical quenching agent. An eighth vial received fluor only and was used for background counts. A standard curve of channels ratio vs. percent efficiency was plotted.

Under the conditions of this assay, homogenate concentrations of fly heads and headless flies were linear functions of the reaction rate (Figures 3 and 4). Figure 5 shows similar results for ^{14}C-AChCl concentration.

Assay for Susceptibility of Cholinesterase to Inhibition

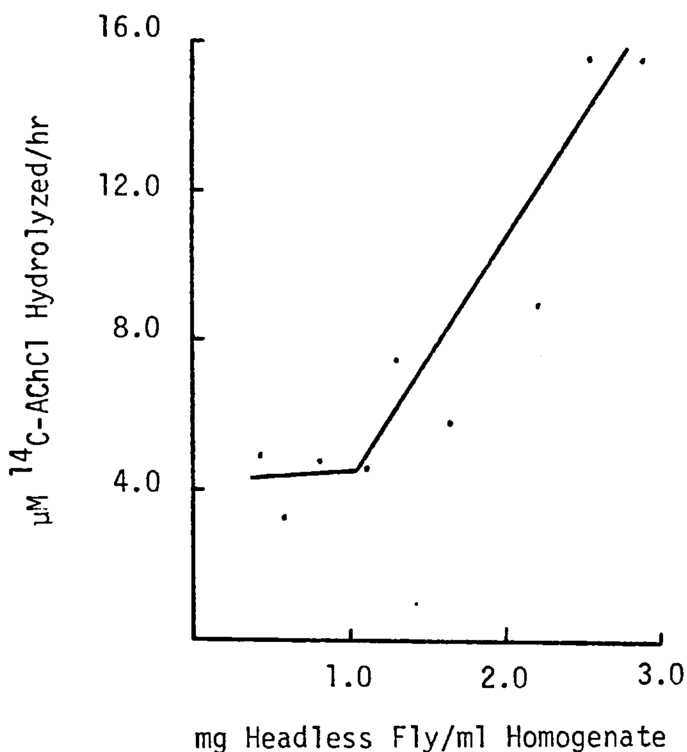
The modified radiometric method of Gaballah (1968) was used to assay for susceptibility of ChE to inhibition by malathion or malaoxon. In the first 6 replicates of the malathion assays, 4 fly heads and 6 headless flies of both sexes, irradiated and non-irradiated, were homogenized. In all other inhibition assays, 6 heads and 8 thoraces were used. The mixture which was incubated at 37°C for 5 minutes consisted of 100 μl homogenate, 100 μl 4mM ^{14}C-AChCl (0.4 μCi/100 μl), and 25 μl of an aqueous 10 ppm malathion or malaoxon solution. A mixture with boiled homogenate was used as control for non-enzymatic hydrolysis of ^{14}C-AChCl, and 2 mixtures without organophosphate insecticides were used to compare the results with typical values of ChE activity levels.



$r = 0.9723$
Significant at 1% Level
$y = 56.4548x + 3.8034$

Figure 3. Cholinesterase activity in heads of male flies as a function of final homogenate concentration in the radiometric assays.

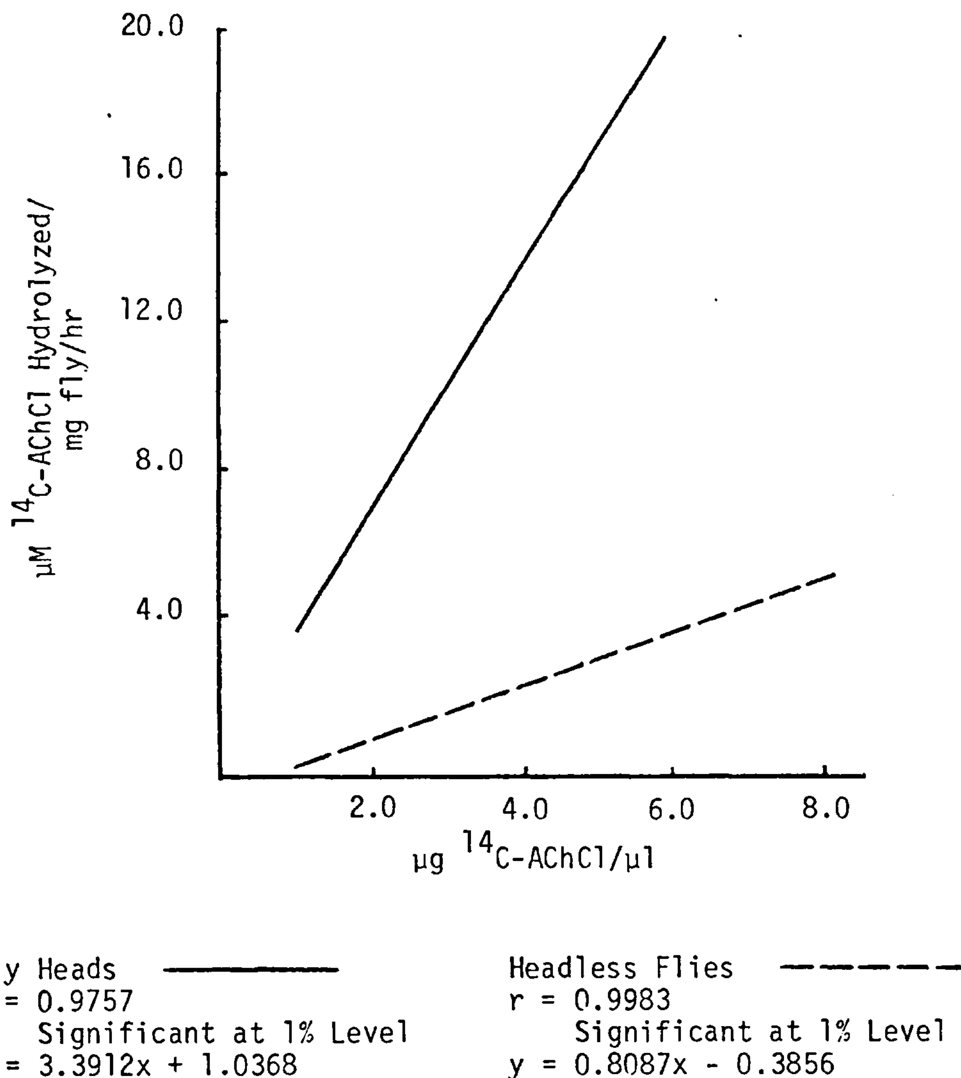
Acetylcholine chloride (^{14}C-AChCl) was present at a final concentration of 3.1 μg/μl.



For $x \geq 1.28$
$r = 0.8384$
Significant at 10% Level
$y = 5.1317x + 0.8875$

Figure 4. Cholinesterase activity in headless male flies as a function of final homogenate concentration in the radiometric assays.

Acetylcholine chloride (^{14}C-AChCl) was present at a final concentration of 3.1 μg/μl.



Fly Heads ————————
r = 0.9757
Significant at 1% Level
y = 3.3912x + 1.0368

Headless Flies – – – – – –
r = 0.9983
Significant at 1% Level
y = 0.8087x - 0.3856

Figure 5. Cholinesterase activity in heads of male flies and headless male flies as a function of final acetylcholine chloride (^{14}C-AChCl) concentration in the radiometric assays.

The homogenate of headless flies was present at a final concentration of 23 mg/ml, and the concentration of fly head homogenate was 2.8 mg/ml.

RESULTS AND DISCUSSION

Level of Cholinesterase Activity

The results of the colorimetric and radiometric assays of ChE activity in flies irradiated as pupae are shown in Table 2. There was no significant difference at the 95% level between activity values for irradiated and non-irradiated, male and female flies in any variation of the assay, nor was there any statistical difference among values for the 3 radiation dosages. The activity values are expressed as μM AChCl hydrolyzed per mg fly per hour. In the colorimetric assay, the values for each replicate represent the average of 2 sub-replicates.

The average value for the colorimetric assay involving head ChE and 6,250 R was 2.82 μM/mg/hr, and the average value for all whole fly colorimetric assays was 0.66 μM/mg/hr. The average value for the whole fly radiometric assay was 0.19 μM/mg/hr. The high activity in the fly heads was expected because of the high activity of ChE in brain tissue.

The values of ChE activity measured by several authors using different methods (Metcalf and March, 1950; Metcalf, March, and Maxon, 1955; Wolfe and Smallman, 1956; Bigley and Plapp, 1960; and Winteringham, 1966) are compared in Table 3 with the values of this study. The values obtained by those investigators ranged from 6×10^{-5} to 3.3×10^{-2} μM/mg/hr.

Table 2. Level of cholinesterase (ChE) activity in house flies X-irradiated as pupae.

Radiation Dosage	ChE Source	Number of Replicates	Acetylcholine Chloride Hydrolyzed µM/mg/hr — Males Control	Males Irrad.	Females Control	Females Irrad.
10,000 R	Whole flies[a]	3	0.21	0.21	0.16	0.20
6,250 R	Whole flies[b]	9	0.74	0.76	0.59	0.63
10,000 R	Whole flies[b]	2	0.74	0.68	0.67	0.48
15,000 R	Whole flies[b]	3	0.55	0.83	0.67	0.61
6,250 R	Heads[b]	6	3.13	3.07	2.62	2.49
6,250 R	Headless flies[b]	2	0.26	0.23	0.19	0.18

a) radiometric assay

b) colorimetric assay

Table 3. Typical *in vitro* cholinesterase (ChE) activity values of house flies as measured by several authors.

Reference	Assay Method	ChE Source	Acetylcholine Choloride Hydrolyzed
Metcalf and March (1950)	manometric	heads	11.0 μM/mg brain/hr
Metcalf, March, and Maxon (1955)	manometric	whole flies	$3.8\text{x}10^{-1}$ μM/mg/hr
Wolfe and Smallman (1956)	titrimetric	heads	$3.0\text{x}10^{-2}$ μM/head/min
Bigley and Plapp (1960)	colorimetric	heads	$7.1\text{x}10^{-2}$ μM/head/min
Winteringham (1966)	radiometric	heads	$6.1\text{x}10^{-5}$ μM/head/min
Present Project	colorimetric	heads	2.8 μM/mg/hr
	colorimetric	whole flies	$6.6\text{x}10^{-1}$ μM/mg/hr
	radiometric	whole flies	6.6 μM/mh/hr

The colorimetric assay has been described as being accurate, consistent, and simple (Witter, 1963), but in this study, the results were often inconsistent among replicates, and the assay was difficult to work with. Errors could have occurred in the numerous pipetting steps of the assay, but the greatest sources of error were probably the rapid fading of color and the adhesion of bubbles to the sides of the cuvettes in the final step of the assay. The fading made precise timing of the spectrotometer readings necessary, and the bubbles had to be removed with a fine brush.

The radiometric assay was simpler and more reliable. The preparation of the homogenate was less time consuming than in the colorimetric assay because of the very low volumes required; however, the problems of uniform mixing of solutions and loss by evaporation were more critical.

When assays of whole flies showed no significant difference in ChE activity between irradiated and non-irradiated flies, it was thought that a small change in activity in the thorax or abdomen might have been masked by the greater quantity of ChE in the head. However, when headless flies were assayed separately, there was still no significant difference in activity. Several radiation levels were used in the assays in order to investigate the possibility that 10,000 R was below a threshold level for ChE alteration or at a level causing massive changes in the fly enzymes. No significant difference in ChE activity was found among the flies irradiated with different dosages. Guenthner and Ware (1967) and Whitacre and Ware (1970) found a

difference in malathion toxicity to male and female flies, but in the present investigation no significant differences between the sexes in the level of ChE activity could be found.

Booth and Metcalf (1970) showed that the primary areas of ChE inhibition by organophosphate insecticides in the house fly were peripheral regions of the thoracic ganglion. Although the radiation-induced changes in the toxicity of malathion and malaoxon do not seem to result from changes in the total level of ChE following irradiation, this does not exclude the possibility that local changes in ChE activity in a critical area of the nervous system may have been masked. A histochemical demonstration of ChE activity, using techniques similar to those of Booth and Metcalf (1970), would be useful in investigating this possibility.

Susceptibility of Cholinesterase to Inhibition

The results of the assays measuring the susceptibility of ChE to inhibition by malathion and malaoxon are shown in Table 4. There was no significant difference at the 95% level in the susceptibility of ChE from irradiated or non-irradiated flies to inhibition by malathion or malaoxon. This was true whether the ChE source was fly heads, thoraces, or headless bodies, or from males or females. As expected, malaoxon inhibited ChE more than did malathion, and ChE from fly heads showed more activity than from other anatomical areas. The fly homogenates undoubtedly contained some desulfurase, phosphatase, and carboxyesterase, but these enzymes were disregarded,

Table 4. Susceptibility of house fly cholinesterase (ChE) to inhibition by malathion and malaoxon following X-irradiation of pupae with 10,000 R.

Inhibitor	ChE Source	Number of Replicates	Acetylcholine Chloride Hydrolyzed µM/mg/hr			
			Males		Females	
			Control	Irrad.	Control	Irrad.
Malathion	Heads	5	2.09	1.83	2.36	2.06
Malathion	Headless flies	2	0.06	0.13	0.06	0.05
Malathion	Thoraces	3	0.20	0.19	0.16	0.15
Malaoxon	Heads	3	0.18	0.19	0.16	0.15
Malaoxon	Thoraces	3	0.14	0.16	0.40	0.10

because all attempts to detect them under conditions similar to the ChE assays were unsuccessful.

There seemed to be no difference in susceptibility of house fly ChE to inhibition by malathion or malaoxon following irradiation of pupae, but in order for this to be more conclusive, other experiments would have to be conducted. Kinetic studies could be made to determine whether the V_{max} and K_m for inhibited and uninhibited ChE are changed by radiation. I_{50} values could be determined for each variation of the assay. Histochemical studies similar to those of Booth and Metcalf (1970) could be made to determine if there are differences in inhibition of localized ChE after irradiation. The histochemical study is important because the effect of radiation on ChE in vital areas of the nervous system could be determined along with the *in vivo* susceptibility of ChE to inhibition in irradiated flies. As the changes in toxicity of malathion and malaoxon following irradiation were determined by *in vivo* experiments (Guenthner and Ware, 1967; Whitacre and Ware 1970), an *in vivo* study of ChE inhibition would be especially important. The rate of penetration of the insecticide to the target and the rate of insecticide metabolism would have to be considered in an *in vivo* study, but the possible release of ChE inhibitors during homogenization would be precluded (O'Brien, 1967).

As the role of total ChE in the mechanism of the radiation-induced changes in the toxicity of malathion and malaoxon seems to be unimportant, another mechanism is proposed. Whole body irradiation of rats and insects is thought to affect the microsomal enzyme system,

which includes malathion desulfurase, phosphatase and carboxyesterase (Terriere, 1968), and the desulfurase activity of young male rats is known to be affected by whole body or head irradiation (Hietbrink and DuBois, 1966). X-Radiation inhibits the development of the desulfurase system rather than altering the activity of the enzyme (Hietbrink and DuBois, 1964). Major inhibition of desulfurase by radiation, combined with inhibition of the other two enzymes, could explain the results obtained by Guenthner and Ware (1967), Whitacre and Ware (1970), and Ware and Whitacre (1970).

If desulfurase were inhibited by radiation, malathion would be less toxic following irradiation. The inhibition of the degrading enzymes, which would lessen this effect, would be unimportant compared with great decrease in toxicity expected if malathion is not converted to malaoxon (O'Brien, 1967). If desulfurase were inhibited, malaoxon activity would not be greatly affected and, as with malathion, inhibition of phosphatase and carboxyesterase would result in a slight increase in toxicity of malaoxon.

Piperonyl butoxide is also a microsome inhibitor (O'Brien, 1967), and it could exaggerate the results by further inhibiting the desulfurase, phosphatase, and carboxyesterase. Thus, piperonyl butoxide would further increase the toxicity of malaoxon in irradiated flies. If the increase in inhibition of desulfurase with piperonyl butoxide were important enough, it would mask the decreased toxicity caused by inhibition of the degrading enzymes, and malathion would

be less toxic following the combined application of piperonyl butoxide and radiation.

SUMMARY AND CONCLUSIONS

The role of ChE in radiation-induced changes in toxicity of malathion and malaoxon to house flies was investigated. Comparisons were made of the levels of total ChE activity between non-irradiated flies and those X-irradiated as pupae. There was no significant difference at the 95% level between ChE activity in irradiated and non-irradiated flies whether the ChE source was from males or females, fly heads, headless flies, or whole flies. Variation of radiation dosage levels from 5,000 R to 15,000 R produced no difference in ChE activity.

Comparisons were also made of the susceptibility of ChE to inhibition by malathion or malaoxon between flies X-irradiated and non-irradiated as pupae. Again there was no significant difference at the 95% level between ChE activity in irradiated and non-irradiated flies, whether inhibited by malathion or malaoxon, and whether the ChE source was from males or females, fly heads, headless flies or thoraces.

The possibility is discussed that major inhibition of malathion desulfurase by radiation, combined with inhibition of phosphatase and carboxyesterase, could explain the decreased toxicity of malathion and the slightly increased toxicity of malaoxon following irradiation as pupae and the exaggeration of these effects by piperonyl butoxide.

REFERENCES CITED

Anonymous. 1961. The Peet-Grady method. Soap and Chemical Specialities Bluebook. 237-39.

Bigley, W. S., and F. W. Plapp. 1960. Cholinesterase and aliesterase activity in organophosphorus-susceptible and -resistant house flies. Ann. Entomol. Soc. Amer. 53: 360-4.

Booth, G. M., and R. L. Metcalf. 1970. Histochemical evidence for localized inhibition of cholinesterase in the house fly. Ann. Entomol. Soc. Amer. 63: 197-204.

Gaballah, S. 1968. A direct radioisotopic microassay for cholinesterase. Proc. Soc. Exp. Biol. Med. 129: 376-80.

Guenthner, A. W., and G. W. Ware. 1967. Effects of X-irradiation on toxicity of malathion, heptachlor, and Temik to the house fly. J. Econ. Entomol. 60: 369-73.

Hestrin, S. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. J. Biol. Chem. 180: 249-61.

Hietbrink, B. E., and K. P. DuBois. 1964. Influence of X-radiation on development of enzymes responsible for desulfuration of an organic phosphorothioate and reduction of p-nitrobenzoic acid in the livers of male rats. Radiat. Res. 22: 598-605.

Hietbrink, B. E., and K. P. DuBois. 1966. Influence of partial body X-irradiation on development of phosphorothioate oxidase in the livers of male rats. Radiat. Res. 27: 669-75.

Hough, W. S. 1963. Effects of gamma radiation on codling moth eggs. J. Econ. Entomol. 56: 660-3.

Keiser, I., and E. L. Schneider. 1969. Longevity, resistance to deprivation of food and water, and susceptibility to malathion and DDT of oriental fruit flies, melon flies, and Mediterranean fruit flies sexually sterilized with Tepa or radiation. J. Econ. Entomol. 62: 663-6.

Metcalf, R. L., and R. B. March. 1950. Properties of acetylcholine esterases from the bee, the fly and the mouse and their relation to insecticide action. J. Econ. Entomol. 43: 670-7.

Metcalf, R. L., R. B. March, and M. B. Maxon. 1955. Substrate preferences of insect cholinesterase. Ann. Entomol. Soc. Amer. 48: 222-8.

Molloy, F. M. 1961. The histochemistry of the cholinesterases in the central nervous system of susceptible and resistant strains of the house fly, Musca domestica L., in relation to diazinon poisoning. Bull. Entomol. Res. 52: 667-71.

O'Brien, R. D. 1967. Insecticides, action and metabolism. Academic Press. New York. 882p.

Rush, R. E., and G. W. Ware. 1969. Effects of gamma radiation on toxicity of DDT, carbaryl and azinphosmethyl to pink bollworm adults. J. Econ. Entomol. 62: 1124-5.

Terriere, L. C. 1968. Insecticide-cytoplasmic interactions in insects and vertebrates. Ann. Rev. Entomol. 13: 75-98.

Varzandeh, M., and W. S. Moos. 1963. The effect of X-radiation on longevity, emergence and DDT-susceptibility of the house fly. Proc. North Cent. Branch Entomol. Soc. Amer. 18: 55-9.

Vashkov, V. I., and V. D. Poleshchuk. 1966. The effect of X-radiation on the insect sensitivity to insecticides. J. Microbio. Epidemiol. Immunobiol. 43: 9-11.

Ware, G. W., and C. K. Irwin. 1969. Conversion of a therapy X-ray unit for insect irradiations. J. Econ Entomol. 62: 721-2.

Ware G. W., and G. K. Whitacre. 1970. Synergist effect of X-irradiation toxicity of malathion and malaoxon to house flies. J. Econ. Entomol. (in press).

Whitacre. G. K., and G. W. Ware. 1970. Effects of gamma- and X-irradiation on the toxicity of malathion to house flies. J. Econ. Entomol. 63: 424-6.

Winteringham, F. P. W. 1966. Metabolism and significance of acetylcholine in the brain of the adult house fly, Musca domestica L. J. Insect Physiol. 12: 909-24.

Witter, R. F. 1963. Measurement of blood cholinesterase. Arch. Environ. Health. 6: 537-63.

Wolfe, L. S., and B. N. Smallman. 1956. The properties of cholinesterase from insects. J. Cell. Comp. Physiol. 48: 215-36.

Wolfenbarger, D. A., and H. M. Graham. 1970. Toxicity of insecticides to irradiated and nonirradiated moths of pink bollworm. J. Econ. Entomol. (in press).

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