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The Induction of Detoxifying Enzymes in Insects

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Microsomal oxidases and DDT-dehydrochlorinase activity are increased in houseflies treated with insecticides such as aldrin, dieldrin, and DDT, the barbiturate phenobarbital, and the insect hormones or hormone analogs such as ecdysone and juvenile hormone. There is evidence that in housefly strains resistant to insecticides by virtue of increased detoxication activity, inducing chemicals cause greater increases in enzyme activity than in susceptible strains treated similarly. It is postulated that this is due to the presence, in the resistant strains, of multiple sets of genes coding for the induced enzymes. The possibility that induction is, or has been, a factor in insect control is discussed. It is concluded that because of the high doses required, at least in the cases examined so far, there is little likelihood that induction has been a factor.

The phenomenon of induction is an appropriate topic of discussion at this symposium on the biochemistry of resistance because in some respects it resembles resistance. Shortly after various species of animals are treated with inducing chemicals the activity of certain enzyme systems is increased, sometimes as much as 25-fold. In this respect such animals are similar to insecticide-resistant insects which are often resistant because they can metabolize the insecticide rapidly enough to escape its toxic effects. Since some of the very insecticides to which insects are now resistant have also been found to be inducers of the detoxication enzymes, it is not surprising that some investigators have wondered whether there is a connection between these two events, *i.e.*, whether induction by these chemicals has been a factor in their ability to select the resistant population.

Probably the first reports of induction in insects were those of Agosin and coworkers (Agosin and Dinamarca, 1963) who observed the phenomenon in *Triatoma infestans*, a blood-sucking insect, when this species was treated with DDT. They found that DDT increased the level of NADP, which is an important cofactor in microsomal oxidation. Later, they showed that the increased level of NADP following DDT treatment resulted from increased activity of NAD-kinase (Ilevicky *et al.*, 1964). These workers suggested that, since DDT was metabolized in some species by the microsomal oxidases (Agosin *et al.*, 1961), its induction of this system might be related to DDT resistance. In a series of papers that followed this discovery, these investigators established that the DDT-stimulated increase in enzymes involved the synthesis of new (*i.e.*, additional) protein (Agosin *et al.*, 1965, 1967), that RNA synthesis was involved (Balazs and Agosin, 1968; Litvak *et al.*, 1968), and that DDT metabolism was indeed more rapid in treated insects (Agosin *et al.*, 1969).

Turning next to a study of the phenomenon in the housefly (*Musca domestica*) Gil *et al.* (1968) obtained results similar to those with *T. infestans* but noted that only certain strains of resistant houseflies, *i.e.*, those with resistance which involved oxidative mechanisms, were inducible with DDT. In several of their reports, the Chilean workers suggested a connection between induction by

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DDT and cross-resistance to other insecticides, noting that the increased enzyme activity would protect the insect from other toxicants which were detoxified by the same enzyme systems. It was also suggested that induction might be involved in the development of resistance, although the nature of this association was not clearly defined.

Oppenoorth and Houx (1968) also attempted to demonstrate induction in resistant strains of the housefly but were unsuccessful with doses of DDT as high as 1 μg/insect. Another early report in which an inducing drug was tested against insects was that of Chakraborty and Smith (1967), who were unable to demonstrate increased detoxication in locusts (*Schistocerca gregaria*) treated with phenobarbital or 3,4-benzopyrene. Unpublished reports from other laboratories indicated that others were having difficulty in demonstrating this phenomenon in insects, although various species of mammals were being found to be susceptible to the effect of a wide variety of chemicals including several insecticides (Conney, 1967). It seems likely now that these early failures with insects resulted from inadequate doses or exposure periods. It will be recognized that dose limitations would be severe when the inducers are toxic insecticides.

More recently there have been reports of induction in houseflies exposed to aldrin and dieldrin (Walker and Terriere, 1970), other cyclodiene compounds (Yu and Terriere, 1971a, 1972), DDT and dieldrin (Plapp and Casida, 1970), phenobarbital, butylated hydroxytoluene, and triphenylphosphate (Perry *et al.*, 1971), juvenile hormone and its analogs (Terriere and Yu, 1973; Yu and Terriere, 1971b), ecdysone (Yu and Terriere, 1971b), and naphthalene (Capdevila *et al.*, 1973a). There is also evidence of induction in the wax moth (*Galleria mellonella*) treated with chlorcyclizine, aminopyrine, and phenobarbital (Ahmad and Brindley, 1969, 1971), in the silkworm (*Hyalophora gloveri*) treated with the juvenile hormone (Whitmore *et al.*, 1972), in the southern armyworm (*Prodenia eridania*) treated with various methyl-substituted benzenes (Brattsten and Wilkinson, 1973), and in the German (*Blattella germanica*) and American (*Periplaneta americana*) cockroaches treated with DDT and dieldrin (Khan and Matsumura, 1972). In most of these reports the enzymes being induced were the microsomal oxidases and in some cases DDT-dehydrochlorinase, but the report by Whitmore *et al.* (1972) deals with increased esterase activity. The number of reports and the growing divergence of species indicate that induction in insects, as in mammals, will be found to be a common phenomenon.

It was the early reports of Gil *et al.* (1968) and of Oppenoorth and Houx (1968) that induction could not be demonstrated in certain strains of the housefly which caught our attention. Being involved in the study of resistance in the housefly, we were interested in any evidence of biochemical differences between R and S or between various R strains. The suggestions that induction and resistance were related phenomena were also intriguing. These aspects of induction led us to begin our own study of it.

Induction with the Cyclodiene Insecticides. Our first experiments were with dieldrin applied to three strains of dieldrin-resistant houseflies, each with a different level of baseline microsomal oxidase (mfo) activity. We attribute the dieldrin resistance to the factor on chromosome IV which appears to be nonmetabolic in nature, *i.e.*, it does not involve the microsomes. The strain with the highest mfo activity, Isolan-B, is resistant to DDT and to carbamates as well as dieldrin. Its carbamate resistance is due to its high oxidase activity and its DDT resistance to its DDTase activity. Its microsomal oxidase activity at the time was about ten times that of a normal susceptible strain.

The Orlando-DDT strain, resistant to DDT by virtue of DDTase, has an intermediate level of mfo activity about three to five times the normal level.

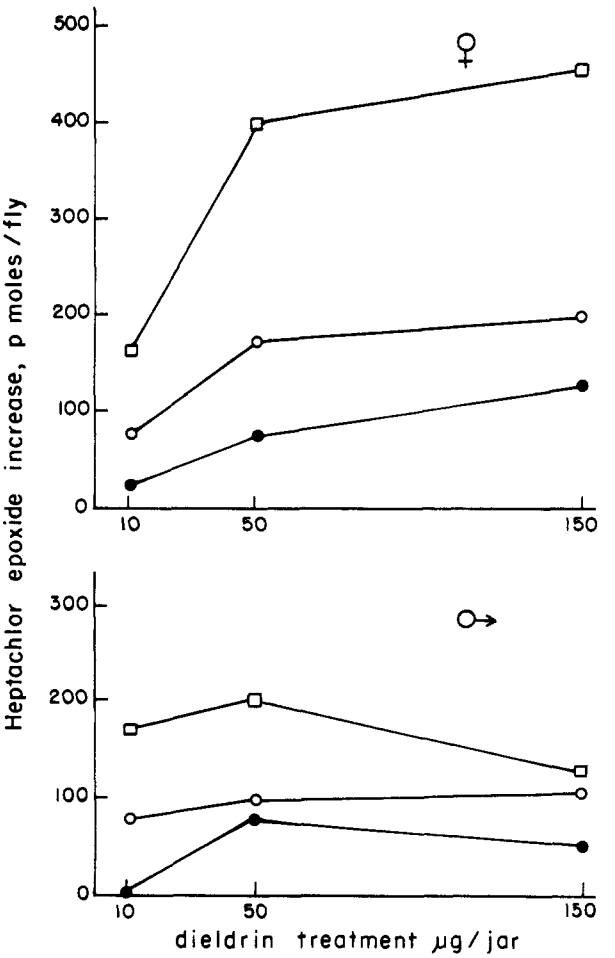

Figure 1. Increase in microsomal heptachlor epoxidase activity in dld;cyw (●), Orlando (○), and Isolan (□) houseflies exposed to different levels of dieldrin. Females, 4 days, and males, 5 days old, were exposed to dieldrin in pint jars for 24 hr prior to enzyme assays. Values of control flies have been subtracted to show net increase in epoxidase production (Walker and Terriere, 1970).

The third strain was the dieldrin-curly wing (dld;cyw). Except for its resistance to dieldrin, it is normal in susceptibility to other compounds and its oxidase level is low, equal to or less than that in normal susceptible strains.

The insects were exposed to dieldrin in two ways, by topical application to the dorsal thorax and by 24-hr exposure to deposits of the inducer in glass jars. Since both topical and tarsal contact treatment methods gave similar results, we used the tarsal contact most of the time, due to its simplicity. We later tried feeding dieldrin, as others have done, and obtained similar levels of induction.

Examples of the results of these early experiments are shown in Figures 1 and 2. It will be seen that the phenomenon has both a dose and a time effect, the optimum doses being about 50 μg/jar and the maximum increase in enzyme activity occurring after 18 to 24 hr exposure. It should be noted that the inducing dose of 50 μg/jar is approximately 100 times the dose which is normally toxic to houseflies. No induction was obtained at doses of 5 μg/jar. These results help explain why induction is not easily demonstrated in S insects.

It will be noted that the enzyme values are plotted as net increases, *i.e.*, the difference between the control level for the strain and its induced level. When this is done, the R (high oxidase) strains are seen to exhibit a much greater

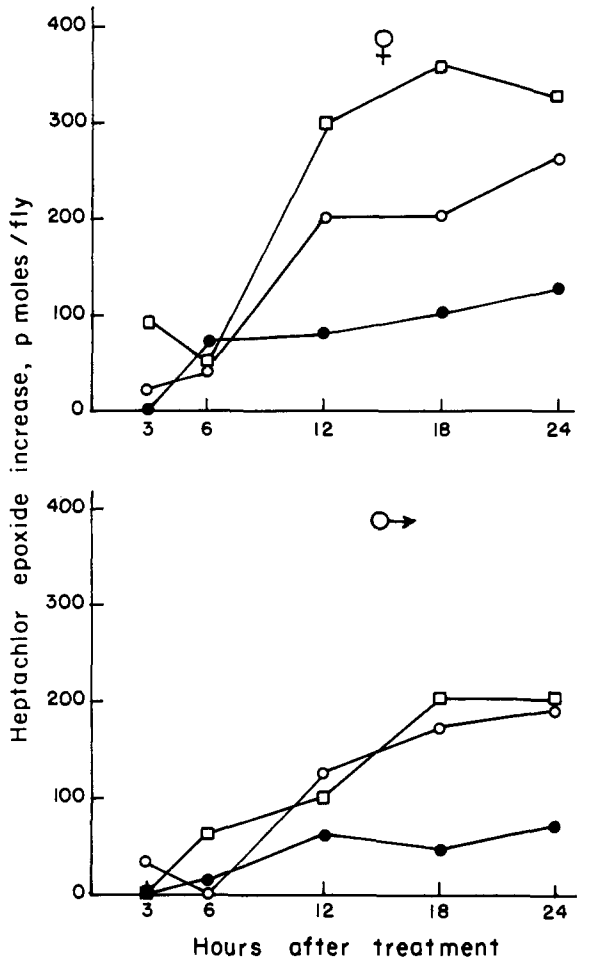

Figure 2. Rate of increase in microsomal heptachlor epoxidase activity in dld;cyw (●), Orlando (○), and Isolan (□) houseflies treated topically with 0.5 μg of dieldrin/fly. Flies were 3 days old. Values of control flies have been subtracted to show net increase in epoxide production (Walker and Terriere, 1970).

response to the inducer. This occurs, as well, when the flies are repeatedly exposed to dieldrin and observed over a period of 20 days. Such treatments were made to allow for the fact that these three strains are known to reach the maximum baseline level of microsomal oxidase activity at different ages. Thus, whether the inducer is applied in a single dose and the time response noted in a continuous dose and the degree of induction noted or in multiple doses and the persistence of the effect noted, the results are essentially the same: the high oxidase strain shows the greatest net increase and the low oxidase strain the least increase.

An explanation of these results is that the R flies possess more sites for regulation, more sources of the regulatory substances (the repressor), and, consequently, are more susceptible to the effects of an inducer. This idea is diagrammed in Figure 3. It can be viewed as a matter of gene titer, the high oxidase strain having several sequences of mfo genes or gene complexes. This would explain the higher baseline level of mfo activity in the normal untreated fly and, on induction, the greater increase in enzyme activity.

We tested this hypothesis by producing hybrids of the high and low oxidase strains and comparing their enzyme activities before and after induction with those of the parent strains. It was reasoned that if both parent strains possessed the normal (S strain) regulatory mechanisms, the hybrids would be induced to the same extent as the S parent, assuming regulation and its disruption by the inducer to be the limiting factors. If the regulator system of

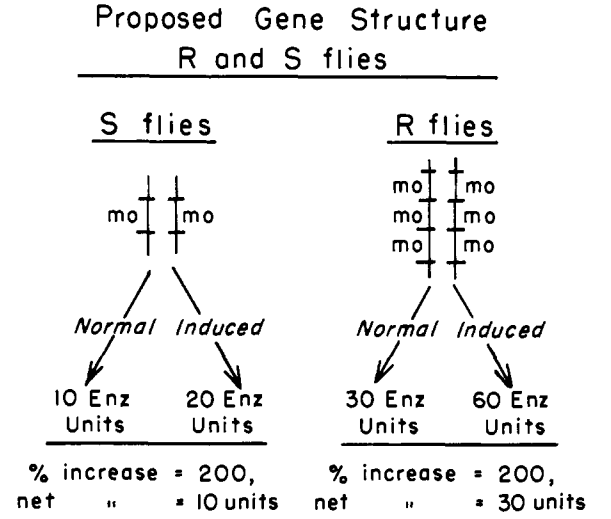

Figure 3. Diagram of hypothetical arrangement of the genes for microsomal oxidases (MO) in R and S houseflies and the amount of oxidase activity resulting in the normal and induced condition.

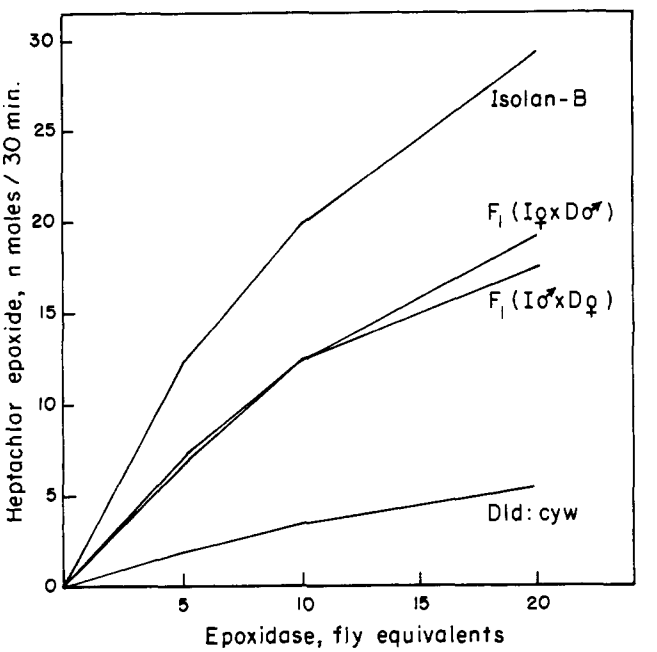

Figure 4. Effect of enzyme level on heptachlor epoxidase activity of microsomes of dieldrin-induced parents and their F_1 progeny. Females, 9 days old (Terriere *et al.*, 1971).

the R strain permits a wider range of enzyme activity, we expected the hybrids to be induced to a greater extent than the S parent.

The results of these experiments are summarized in Table I and Figure 4. They support the theory that the strains differ in degree of regulation and are thus consistent with the notion that the R strain is redundant in the genes controlling the mfo enzymes.

CD Induction of *O*-Demethylase. Although by this stage of our work we had shown that the CD compounds were inducers of naphthalene hydroxylase and of heptachlor epoxidase (Walker and Terriere, 1970), we tested still another of the reactions performed by the microsomes, O-demethylation using *p*-nitroanisole as substrate. The results of some experiments with this system in the Isolan-B and dld;cyw strains are shown in Table II.

Comparing the effects of aldrin and dieldrin on the two strains, we see that the Isolan-B strain is induced to a net increase of 36 and 25 pmol compared to 15 and 10 pmol,

Table I. Dieldrin Induction of Microsomal Heptachlor Epoxidase Activity in High and Low Oxidase Parents and Their F_1 Hybrids[a]

	Heptachlor epoxide, pmol/fly/30 min			
	Flies 3 days old		Flies 7 days old	
Housefly strains[b]	Control	Treated	Control	Treated
Dld:cyw	32.2	129.9	42.5	131.5
F_1(I♂ × D♀)	146.7	252.8	193.9	452.4
F_1(I♀ × D♂)	144.2	279.8	129.0	350.6
Isolan-B	311.3	898.9	378.6	707.3

[a] Terriere *et al.* (1971). [b] Female flies exposed to dieldrin (50 μg) in pint glass jars for 24 hr prior to enzyme assay.

Table II. *O*-Demethylase Activity in Houseflies Treated with Cyclodiene Insecticides[a]

			O-Demethylase activity	
Housefly[b] strain	Insecticide	Dose, μg/jar	*p*-Nitrophenol, pmol/fly/min	Increase, pmol
Isolan-B	Untreated	0	44.93 ± 1.80	
Isolan-B	Aldrin	150	80.87 ± 8.98	36
Isolan-B	Dieldrin	75	70.09 ± 1.80	25
Dld;cyw	Untreated	0	9.53 ± 1.25	
Dld;cyw	Aldrin	150	25.16 ± 1.80	15
Dld;cyw	Dieldrin	75	20.06 ± 0.62	10

[a] Yu and Terriere (1972). [b] Three-day-old Isolan-B females, 24 hr exposure.

respectively, in the low oxidase strain. Thus, as before, the high oxidase strain not only has a higher baseline level of this enzyme's activity but is also induced approximately twice as much.

Induction with Phenobarbital (PB). A major drawback of the cyclodiene compounds as inducers is their toxicity, permitting their use only on CD-resistant strains. This introduced the complication that all of the strains studied had an additional factor or factors of unknown influence on the results. This problem was overcome when it was reported that phenobarbital, long known as an inducer in vertebrates, was equally effective with houseflies and *Triatoma infestans* (Agosin *et al.*, 1969; Perry *et al.*, 1971).

This inducer allowed the use of susceptible flies such as the WHO-SR strain with which to compare other strains. An example of our results with phenobarbital as inducer of the epoxidase system is shown in Figure 5. Here we see the effect of inducer level on enzyme activity. Doses above 1% PB in the diet were not tolerated well by the SR strain, hence the termination of the plot at this point. The potency of this inducer is shown by the large increase in enzyme activity, nearly 25-fold in the SR strain. An important difference from the previous results with the CD compounds is that the low oxidase strain was induced to a greater extent than the R strain. This is not consistent with our theory of gene redundancy.

We reasoned that the greater effect of PB on the S than on the R strain after 3 days of exposure was due to the initially higher enzyme activity of the R strain. On inducing these enzymes to still greater activity, they might be able to metabolize the inducer sufficiently to reduce its internal concentration below the optimum for full induction. When enzyme activity was measured at 0, 1, 2, 3, and 4 days, we obtained the results shown in Figure 6. At day 1, the Isolan-B strain was induced twice as much (40 pmol compared to 20 pmol) as the SR strain. This advantage was soon lost, the net increase in enzyme activity being greater for the S strain at 2 days and thereafter,

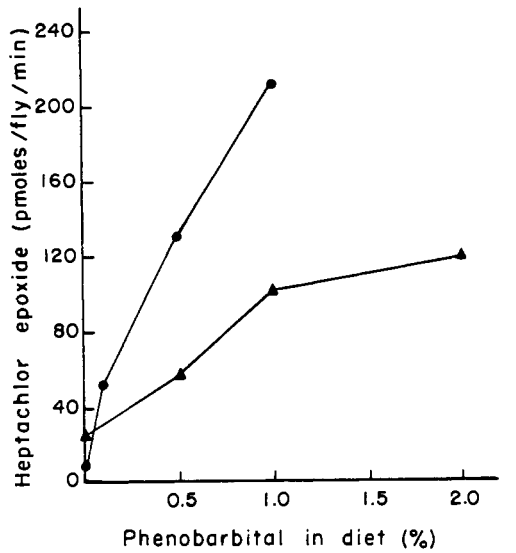

Figure 5. Microsomal heptachlor epoxidase activity in a high (Isolan-B) (▲) and a low (SR) (●) oxidase strain fed varying levels of phenobarbital for 3 days. The female adult flies were 3 days old when the feeding was started (Yu and Terriere, 1973).

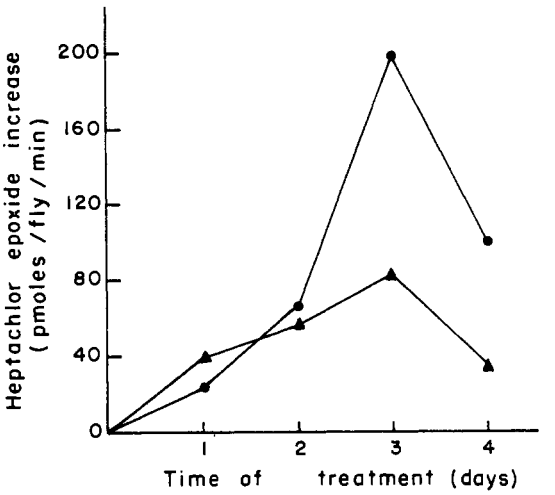

Figure 6. Net increase in microsomal heptachlor epoxidase activity in a high (Isolan-B) (▲) and a low (SR) (●) oxidase housefly strain fed 1% phenobarbital for 1 to 4 days. The female flies were 3 days old when the treatments began (Yu and Terriere, 1973).

supporting the belief that the inducer may have been metabolized more rapidly in the high oxidase strain. We also found that the S strain is more sensitive to PB, 0.1% in the diet causing a 61% increase in epoxidase activity, while this dose had no effect on the Isolan-B strain.

In these experiments we also measured some other components of the mfo system as it was under induction by PB. Table III shows that cytochrome c reductase and cytochrome P-450 were increased 20 to 50%, while the epoxidase activity rose by nearly 200%. This result is not uncommon, as several other workers have observed a much larger induction of the overall oxidase activity than of the P-450 component.

Our results with PB induction of *p*-nitroanisole *O*-demethylase are shown in Table IV. Again, at 3 days after PB feeding began, the SR strain was induced to a greater extent than the Isolan-B strain. We suggest, as before, that this is due to the metabolism of the inducer by the more active strain, thus reducing its impact. It seems possible that when dieldrin is the inducer, its stability to mfo attack prevents this bias and we see both epoxidase and

Table III. Effect of Induction by Phenobarbital on Microsomal Electron Transport Components[a]

Treatment[b]	Cytochrome c red., ΔOD × 10^3 min/abd	Cytochrome P-450, pmol/abd	Heptachlor epoxide, pmol/abd/min
Control	36.15 ± 0.15	15.40 ± 0.79	23.00 ± 2.31
Phenobarbital (1%)	42.70 ± 0.70	23.69 ± 3.69	60.41 ± 1.29

[a] Yu and Terriere (1973). [b] Phenobarbital in diet for 3 days. Isolan-B females were 3 days old when treatment was begun.

Table IV. Microsomal *O*-Demethylase Activity in Houseflies Fed Phenobarbital[a]

	p-Nitrophenol, pmol/fly/min	
Treatment[b]	SRS	Isolan-B
Control	27.06 ± 1.41	49.12 ± 1.04
Phenobarbital (1%)	126.99 ± 1.52	80.75 ± 1.92

[a] Yu and Terriere (1973). [b] Phenobarbital in diet for 3 days. Female adults were 3 days old when treatment was begun.

Table V. DDT-Dehydrochlorinase Activity in Houseflies Treated with Cyclodiene Insecticides[a]

			DDT-Dehydrochlorinase	
Housefly[b] strain	Insecticide	Dose, μg/jar	DDE, pmol/fly/min	Increase, pmol
Isolan-B	Untreated	0	100.93 ± 5.0	
Isolan-B	Aldrin	150	176.03 ± 11.6	75
Isolan-B	Dieldrin	75	140.28 ± 3.0	39
Dld;cyw	Untreated	0	19.44 ± 3.2	
Dld;cyw	Aldrin	150	46.01 ± 5.6	27
Dld;cyw	Dieldrin	75	39.95 ± 5.5	21

[a] Yu and Terriere (1972). [b] Three-day-old females, 24 hr exposure.

demethylase activity increased more substantially in the R strain.

In vertebrates, where induction has been extensively studied, it has been possible to classify inducers into two groups, those which stimulate the production of cytochrome P-450 and those which stimulate production of another species, variously known as P-446, P-448, or P_1-450. Phenobarbital is a member of the first group, while 3-methylcholanthrene (3-MC) belongs to the second. We have tried in various ways to obtain induction in houseflies by treatment with 3-MC, all without success. This has included applications by injection, topical, feeding, and tarsal contact, with treatment periods ranging up to 3 days. In no case was there the slightest stimulation of microsomal epoxidase or *O*-demethylase activity. We conclude that 3-MC is not an inducer of these enzymes in the housefly.

There is now evidence (Capdevila *et al.*, 1973b) that naphthalene and phenobarbital induce a new species of P-450 in the housefly (F_c strain), the CO difference spectrum of the hemoprotein having a maximum absorption at 446 nm. In this respect the result is similar to that obtained with 3-MC as the inducer in rats.

Induction of DDTase. Most of the attention given to induction of drug-metabolizing systems has centered on the microsomal oxidases, these being the enzymes involved in the metabolism of the inducing drugs. As shown in Table V, DDT-dehydrochlorinase activity is also increased after treating houseflies with various CD compounds.

It will be noted that the Isolan-B strain has about 5× as much baseline DDTase activity as the low oxidase strain. This resistance is due to dehydrochlorination, not microsomal oxidation. The inducing agents increased the enzyme activity approximately twofold in both strains, the percentage increase being a bit more in the dld;cyw strain. Using the other yardstick, net increase in enzyme activity, it can be seen that, as with the oxidases, the strain with the highest baseline activity is induced to the greatest extent. The net increase for aldrin at 150 μg/jar is 75 and 27 pmol, respectively, for the high and low oxidase strains.

The results with phenobarbital as inducer of DDTase in the Isolan-B and SR strains are even more striking. The SR strain was developed as a baseline strain, susceptible to insecticides, to be used as a reference strain in resis-

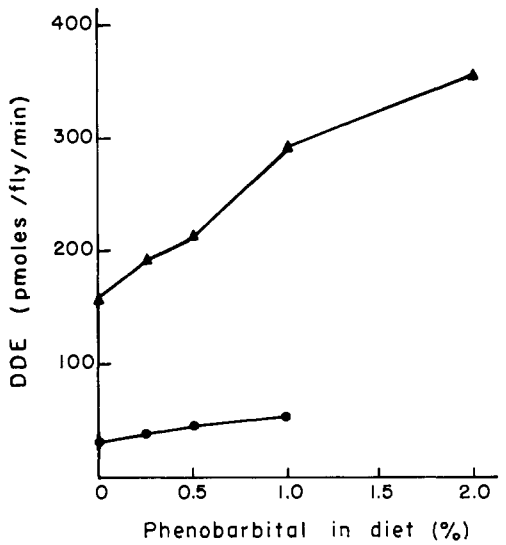

Figure 7. DDT-dehydrochlorinase activity in a high (Isolan-B) (▲) and a low (SR) (●) oxidase strain fed varying levels of phenobarbital for 3 days. The female adult flies were 3 days old when the feeding was started (Yu and Terriere, 1973).

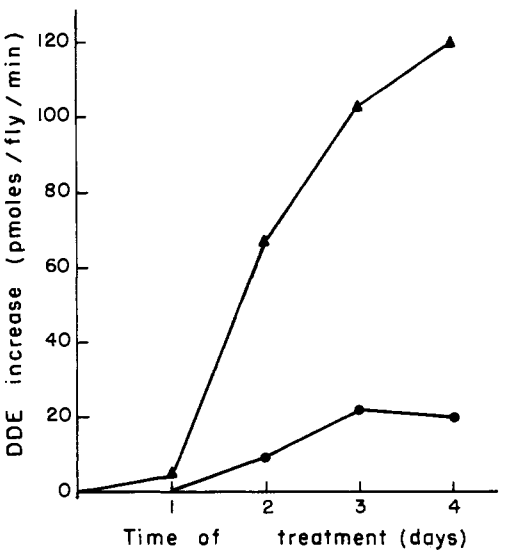

Figure 8. Net increase in DDT-dehydrochlorinase activity in a high (Isolan-B) (▲) and a low (SR) (●) oxidase housefly strain fed 1% phenobarbital for 1 to 4 days. The female flies were 3 days old when the treatments began (Yu and Terriere, 1973).

Table VI. Induction of Detoxifying Enzymes in Houseflies Fed the Juvenile Hormone and Some of Its Analogs[a]

JHA in diet,[b] %		Housefly strain	Enzyme activity, pmol/fly/min[c] Heptachlor epoxidase	*p*-Nitroanisole *O*-demethylase	DDT–Dehydrochlorinase
Control		Isolan-B	24.72 ± 0.86	49.23 ± 2.52	142.85 ± 4.28
JH[d]	0.25	Isolan-B	36.15 ± 0.81		
	0.50	Isolan-B	41.45 ± 1.00		
	1.00	Isolan-B	44.24 ± 1.32	107.82 ± 2.95	185.22 ± 6.97
Methoprene					
(69%)	0.1	Isolan-B	26.99 ± 0.45		
	0.5	Isolan-B	31.42 ± 1.02		
	1.0	Isolan-B	33.13 ± 0.99	49.42 ± 0.90	152.18 ± 16.37
Hydroprene					
(63%)	1.0	Isolan-B	22.73 ± 1.63	51.93 ± 2.71	140.44 ± 2.10
MDP-JH	0.1	Isolan-B	10.98 ± 0.79	18.14 ± 0.37	176.83 ± 4.16
	0.5	Isolan-B	12.24 ± 0.22	18.33 ± 0.36	217.13 ± 1.86
	1.0	Isolan-B	12.68 ± 0.57	15.10 ± 0.71	280.20 ± 4.51
Piperonyl butoxide	1.0	Isolan-B	17.65 ± 0.40	37.75 ± 0.72	290.21 ± 1.04
Control		SRS	6.46 ± 0.72	32.95 ± 1.51	26.60 ± 0.59
JH	1.0	SRS	12.13 ± 0.71	43.85 ± 1.48	30.14 ± 1.78

[a] Terriere and Yu (1973). [b] One-day-old female adults fed the diets for 3 days. [c] Mean ± SE of three to four experiments, each with duplicate incubations. [d] JH = methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (the Cecropia juvenile hormone); Methoprene = isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate (a mixture of isomers containing 69% trans,trans form); Hydroprene = ethyl 3,7,11-trimethyldodeca-2,4-dienoate (a mixture of isomers containing 63% trans,trans form); MDP-JH = 6,7-epoxy 3,7-diethyl-1-[3,4-(methylenedioxy)phenoxy]-2-octene (a mixture of isomers of unknown ratio).

tance studies. This strain would be expected to have a low level of DDTase activity. Figure 7 shows that in this strain DDTase is only slightly induced by phenobarbital while in the Isolan-B strain induction is extensive. As with the epoxidases, rather heavy doses of phenobarbital are required, the maximum induction occurring at dietary levels of 1% or more. If there is doubt that induction actually occurred in the SR strain, this should be dispelled by Figure 8, where the scale is smaller and the net increases are plotted against time after beginning exposure to the inducer. It is clear that the inducer has a relatively larger effect on the DDT-R strain than on the S strain. A perplexing question in connection with these results is the reason for their variance from the previous experiments in which phenobarbital was the inducer of microsomal oxidases in these same two strains (Figures 5 and 6). If phenobarbital was being metabolized by the microsomes so as to reduce its internal concentration in the Isolan-B strain compared to its level in the SR strain, should we not expect the same "reversal" in enzyme activity with DDTase?

The only answers we can suggest at present are that the DDTase system is more sensitive to phenobarbital, so that its partial metabolism by the microsomes is of no consequence or that the metabolites of phenobarbital are also inducers of DDTase.

Induction by Insect Hormones. The suggestion that insect hormones act as inducers (Karlson and Sekeris, 1962) prompted further experiments with ecdysone and various analogs of the juvenile hormone. As reported elsewhere (Yu and Terriere, 1971b), we found that the microsomal oxidases of the housefly are stimulated by ecdysone treatment. The stimulation was more transient than that produced by dieldrin or PB and the increase in enzyme activity was less. The juvenile hormone analog, derived from farnesenic acid (Law *et al.*, 1966), was also tested and found to stimulate the mfo system. The patterns of stimulation by JH and by ecdysone are somewhat different, with the JH effect coming later.

We have extended these experiments with additional juvenile hormone and JH analogs. From the results summarized in Table VI, it is seen that the JH analogs and the Cecropia JH exert different effects. The Cecropia JH, which is thought to be the natural hormone in some species besides Cecropia, exerted a strong stimulation of the oxidases. This was true of both the Isolan-B strain and the SRS, although the effect on the latter was less.

The JHA methoprene, which has insecticidal properties in certain dipterous species, had a slight enhancing effect on the epoxidase but none on the *O*-demethylase of the Isolan-B strain. A related compound, hydroprene, did not effect either enzyme.

The compound we call MDP-JH because it is an ether of methylene dioxyphenol resulted in the expected inhibition of the two oxidase systems, as did the synergist piperonyl butoxide. More surprising was the stimulation by both compounds of DDTase activity. The Cecropia JH also stimulated this enzyme but neither of the analogs, methoprene or hydroprene, had an effect. This lack of activity as inducers might have something to do with the insecticidal activity of these analogs.

During our tests we discovered that the microsomal oxidases are able to metabolize methoprene and hydroprene and that resistant insects do this at a more rapid rate. This confirms the reports of others that insects may become resistant to these compounds (Cerf and Georghiou, 1972; Dyte, 1972).

Another thought in conjunction with the action of the hormones on microsomes is that hormone stimulation of these enzymes may be a mechanism for the regulation of hormone titer. Thus, oxidation of the JH or of ecdysone could lead to either activation or inactivation of these compounds.

Specificity of Inducers. There is evidence of specificity in the results already shown with the JH analogs as inducers of microsomal oxidases and of DDTase, the Cecropia JH stimulating both systems, hydroprene stimulating neither, and the MDP-JH stimulating DDTase while inhibiting the mfo system.

In a study of induction by five olefin–oxide pairs of cyclodiene compounds (Yu and Terriere, 1972), we observed that the oxides were effective at lower doses, *i.e.*, they were more efficient inducers. The most interesting results of these experiments were those with endrin and isodrin, which are stereoisomers of dieldrin and aldrin, respectively. Although endrin and isodrin are not very active as inducers compared to aldrin and dieldrin, they are effective at about 1/300th the dose.

Effect of Inducers on Cytochrome P-450. The induction of microsomal cytochrome P-450, the terminal com-

ponent of the mfo electron transport chain, has been demonstrated in the housefly by several investigators. Matthews and Casida (1970) found an increased level of cytochrome P-450 in the Orlando-DDT strain of houseflies after treatment with dieldrin. According to them, the dieldrin-induced cytochrome P-450 in females of this strain was qualitatively different from the controls when ethyl isocyanide was the ligand. Perry *et al.* (1971) later showed that phenobarbital, butylated hydroxytoluene, and triphenyl phosphate were inducers of cytochrome P-450 in resistant and susceptible houseflies. In our laboratory, we also observed that the Cecropia juvenile hormone and phenobarbital increased the P-450 content in houseflies (Terriere and Yu, 1973; Yu and Terriere, 1973). In all instances, these authors noted a much greater induction of the microsomal oxidase activity than of the P-450.

An explanation of this result may be found in the recent report of Capdevila *et al.* (1973b), who present evidence that phenobarbital and naphthalene induce a new species of cytochrome P-450 in houseflies and that the induced hemoprotein is more active than the uninduced form.

DISCUSSION

It is clear from years of study of resistance to insecticides in various species of insects that the most important factor in the insect's defensive system is an increased capacity to detoxify the insecticide, most likely as a result of the production of additional enzymes of detoxication. The study of induction in insects has raised the possibility that this increased capacity to detoxify occurs through the regulation of the genes which control the production of such enzymes.

One of the ways in which R and S insects might differ in gene regulation so as to provide the R insects with improved detoxifying ability is that the R insect might be under less regulation. This could occur through a decreased production of the repressor substances (Jacob, 1966), resulting in an increase in the level of detoxifying enzymes. If this were the case, it would be expected that such insects would be less susceptible to the effects of exogenous inducers such as dieldrin or phenobarbital. Our experiments with high and low oxidase strains of the housefly provide evidence against this idea. Indeed, comparing the degree of induction in R and S insects treated with a slowly metabolized inducer such as dieldrin, it is seen that both are induced to a comparable extent from two- to fivefold (Terriere *et al.*, 1971; Walker and Terriere, 1970).

We have postulated that R insects contain more gene sequences for the detoxifying enzymes than their susceptible counterparts (Terriere *et al.*, 1971). This is based largely on the fact that, on induction, the R strains have a greater increase in detoxication capacity. It has been presumed that this represents additional enzymes, since the induction can be offset by suitable treatments with inhibitors of protein synthesis. As shown earlier (Figures 5 and 6), we were unable to repeat these results when phenobarbital was the inducer, possibly because of its rapid metabolism by the very enzymes induced.

The reports of differences in cytochrome P-450 in R and S insects (Matthews and Casida, 1970; Perry *et al.*, 1971; Philpot and Hodgson, 1971; Tate *et al.*, 1973) are not incompatible with the gene redundancy theory, since this idea does not exclude the possibility that the enzymes produced are different.

Another aspect of induction concerns the mode of action of the exogenous chemicals which act as inducers. It has been noted (Orrenius *et al.*, 1969) that the wide variety of chemical structures known to be inducers makes it unlikely that the action is direct. Otherwise, our ideas about the subtleties of gene and enzyme regulation must be in error. More likely, according to this author, there is an indirect action involving an interference with microsomal metabolism of steroid hormones, the actual endogenous inducers. Thus, an exogenous compound which might be a substrate for the microsomal oxidases would be expected to be an inducer by competing with the endogenous steroids for the oxidase enzymes. This could result in an imbalance in steroid titer in the organism with consequent stimulation or inhibition, as the case may be, of genes and of enzyme production.

Practical Aspects of Induction in Insects. One of the reasons for including this topic in this symposium is the possibility, which has been mentioned by some authors, that induction may have had an influence on the development of resistance and that it may still complicate the control of insects by chemical pesticides. The reasoning involved here is that, since these inducers can "turn on" the detoxifying enzymes, they could enhance the already existing detoxification machinery, speed the development of resistance, and cause cross-tolerance to other pesticides. This cannot be denied as a real factor in the laboratory, where we can demonstrate such an effect without question. However, we question its impact under field conditions. These doubts are based on the high doses and long exposures required to achieve induction even under laboratory conditions.

To briefly review the doses we and others have found to be required to show induction, we begin with the work of Agosin and his colleagues (Ilevicky *et al.*, 1964), who were the first to study this problem in insects. These workers used DDT at 300 μg/bug to demonstrate the induction of NAD-kinase in *T. infestans*. We suggest that this is a rather massive dose, not likely to be encountered in the field.

The Agosin group, in their work on induction of houseflies by DDT, found it necessary to use doses, topically applied, ranging from 5 to 10 μg/fly, to bring about increases in detoxifying enzymes. Their recent paper (Capdevila *et al.*, 1973c) reports that 5 μg/fly was the minimum dose inducing DDTase. Since the toxic dose for DDT in flies is about 0.3 μg/fly, we see again that the inducing dose is 15 times higher than the toxic dose. It seems unlikely that S flies exposed to such doses in the field would survive.

Plapp and Casida (1970) and Hodgson and Plapp (1970) report induction with DDT fed to flies at 1000 ppm in their diet, obtaining only modest increases in microsomal oxidase activity, while dieldrin had to be fed at 100 ppm to obtain induction. It is highly doubtful that a fly encounters such doses in the field.

In our hands it has been shown that as a contact residue the CD compounds must be used at 10 to 50 μg/jar for 12–18 hr to demonstrate induction. The toxic dose of these compounds, except for endrin, under these conditions is of the order of 1 μg/jar. With phenobarbital a dose of 1% in the diet seems excessive, again calling attention to the lack of sensitivity of the induction mechanism.

There is still another reason to believe that inducing chemicals will have little effect in the field, either on their own metabolism or in cross-tolerance for other pesticides. This is shown in Table VII, which summarizes our attempts to demonstrate a protective action when phenobarbital is fed to flies prior to their exposure to propoxur. The protective effect was only partial in spite of the fact, as shown earlier, that this drug increases enzyme activity as much as 25-fold.

It should be added that a more potent inducer or a more sensitive species than we have so far found could change the picture. It should also be noted that the hormones might also influence resistance. These are active at much lower doses and, of course, are not toxic. Their action, in stress conditions or in the natural events of

Table VII. Effect of Phenobarbital on the Toxicity of Propoxur to Houseflies[a]

Propoxur, μg/jar	Housefly strain	% mortality in 24 hr at indicated days after withdrawal of phenobarbital[b]			
		0[c]		3[c]	
		Control	Treated	Control	Treated
5.0	SRS	42 ± 7	23 ± 5[e]	56 ± 3	31 ± 3[d]
7.5	SRS	82 ± 5	62 ± 5[e]		
10.0	SRS	96 ± 2	86 ± 3	92 ± 2	86 ± 3
25	Isolan-B	33 ± 5	31 ± 5	37 ± 8	27 ± 6[d]
50	Isolan-B	63 ± 8	60 ± 7	56 ± 6	49 ± 7[e]
100	Isolan-B	77 ± 4	65 ± 7	75 ± 3	63 ± 9[d]
200	Isolan-B			81 ± 4	63 ± 8[d]

[a] Yu and Terriere (1973). [b] Three-day-old females fed a diet containing 1% phenobarbital for 3 days prior to propoxur treatment. Means ± SE of four experiments. [c] The mfo activity (heptachlor epoxidase, pmol/fly/min) of the strains was: 0 day, SRS control, 8.20; treated, 206.86; Isolan-B control, 25.18; treated, 103.01. 3-day, SRS control, 8.95; treated, 36.55; Isolan-B control, 25.23; treated, 35.66. [d] Values significantly different ($p < 0.01$) from the controls. [e] Values significantly different ($p < 0.05$) from the controls.

growth, could well influence the response of an insect to a toxicant.

CONCLUDING REMARKS

To summarize these ideas in the context of the theme of this symposium we wish to emphasize that, although the phenomenon of induction is extremely interesting, its main value here is in the light it may shed on resistance. We must ask the question, "What is the source of the increased detoxication activity?" All the evidence to date shows that the enzymes involved are adaptive in nature, *i.e.*, they are regulated. This being the case, we can ask, "Have these enzymes become constitutive at their highest level of production as a means of providing additional protection against toxic substances?" The answer is "No." Another possibility is that the R enzymes are more active, the result of gene mutation. There is some precedent for this in the case of the aliesterases and their metabolism of OP compounds, but the evidence accumulated so far does not support it with the microsomal oxidases and DDTase. But there is more to be done on this point and it may still be found that the explanation is one of improved activity or stability through mutation. So we must leave this question unanswered.

Another question is, "What other possibilities are there which would explain the increased enzyme activity in the R insect and be compatible with the induction data acquired so far?" The answer which we would provide here is that the R insect has acquired, through genetic aberrations, extra sets of genes coding for the detoxication enzymes. This would provide the selective advantage when toxic substances are present. It would result in a greater supply of enzymes under normal circumstances, as observed, and a greater net increase under stress circumstances, that is, induction by exogenous or endogenous inducers.

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