Hepatic Aminopyrine N-demethylase, Acetanilide Hydroxylase and Lipid Peroxidation in Young Growing Rats during the Treatment of Insecticides

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Halogenated hydrocarbon insecticides and their metabolites are known to alter the activity of microsomal enzymes(HART et al.1963, HART and FOUTS 1963, 1965, WELCH et al.1967, BUNYAN and PAGE 1973). In contrast to the halogenated hydrocarbons there are very few reports regarding the effect of organophosphorus and carbamate insecticides on microsomal drug metabolizing enzymes. Hepatic drug metabolism has been found to be decreased by malathion in mice and rats (STEVENS et al. 1972, RAO and ANDERS 1973, STEVENS and GREENE 1973). Very recently, STEVENS and GREENE (1974) have showed that subacute administration of insecticides resulted in induction of hepatic drug metabolism and mixed function oxidase systems in rats; however, WEBER et al,(1974) have reported that parathion inhibited the metabolism of benzo(a)pyrene in rat liver and intestine. Our earlier investigations have reported an increase in drug metabolizing enzymes and lipid peroxidation during Take-20 administration and a decrease in drug metabolism followed by an increased lipid peroxidation during Baygon administration in young growing rats (MAKHIJA and PAWAR 1974,1975). A literature review indicates a paucity of toxicological data and hence the present experiments were planned to investigate the effect of Take-20 (0,0-dimethyl malathion) and Baygon on hepatic drug metabolism and lipid peroxidation in young growing rats.

MATERIALS and METHODS

Young male and female C.F. strain albino rats initially weighing 55-65 gms were obtained from M/S Ghosh and Co.,Calcutta. The animals were housed in individual cages in an air conditioned room and supplied with standard rat pellets (obtained from Hindustan Lever Ltd., Bombay) and water ad libitum for 10-15 days. The animals were then divided into the following four groups each containing 5-6 animals.

1) Control group of rats.
2) Baygon treated group of rats (10 mg/kg).
3) Take-20 (0,0-dimethyl malathion) treated group of rats (150 mg/kg).
4) Baygon + Take-20 (10 mg + 150 mg/kg) treated group of rats.

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In the injection experiments, the injected and the control animals were pair fed. One group of animals were injected intraperitoneally with Baygon (10 mg/kg) using corn oil as a carrier. Another group of animals were injected with Take-20 (0,0-dimethyl malathion, 150 mg/kg0 and the last group of animals received Baygon and Take-20 in combination. The control animals received only corn oil. All animals were injected for two days, and sacrificed 24 hours after the last injection. The livers were perfused with 0.9% ice-cold saline, removed, blotted dry, weighed, minced and homogenized (1:10 w/v) in ice-cold 0.25 M sucrose with a teflon pestle glass homogenizor. The homogenates were centrifuged at 9000xg for 20 minutes in a Remi K-24 centrifuge. The 9000xg supernatant fraction protein was estimated according to the biuret method (GORNALL et al.1949).

The drug metabolizing enzymes were assayed as reported previously (MAKHIJA and PAWAR 1974). The activities of drug metabolizing enzymes were also estimated in the presence of 2.5×10^{-4} M p-chloromercuribenzoate (PCMB) a commonly used inhibitor of drug metabolizing enzymes. All reactions were carried out at 37°C under air in a Dubnoff metabolic shaker and terminated by addition of 10% ice-cold trichloroacetic acid. The protein free supernatant obtained after centrifugation at 2000xg for 10 minutes was used to estimate the various products formed. The method of NASH (1953) was used to estimate the formaldehyde formed during N-demethylation of aminopyrine. The p-hydroxyacetanilide formed was used as a measure for acetanilide hydroxylase activity as described by WEISBURGER and GOODALL (1968).

The lipid peroxidation was assayed and estimated by the thiobarbituric acid reaction as reported previously (MAKHIJA and PAWAR 1974).

RESULTS

The relative liver weights were increased during intoxication of insecticides. The magnitude of increase was more in male rats as compared to the female rats during Baygon intoxication, however, it was vice versa during Take-20 injections. When both the insecticides were injected in combination the relative liver weights did not show any significant change in the case of male rats, however, a measurable increase was noticed in the case of female rats.

The 9000xg supernatant protein content was decreased by Baygon treatment both in male and female rats (TABLE 1). The protein content was increased significantly in male rats due to Take-20 treatments, however, it was significantly decreased in female rats. The combination of Baygon and Take-20 resulted in a significant decrease in the hepatic (9000xg) protein levels in male

rats, whereas, in female rats it was decreased.

TABLE 1

Effect of insecticides and their combination on relative liver weights and 9000xg supernatant protein content in young growing rats*.

Group	Sex	Relative liver weight (gms)	9000xg supernatant protein content (mg/gm liver)
CONTROL	Male	3.96	179.4
	Female	4.12	204.9
BAYGON	Male	4.02	160.3^{a}
	Female	4.47^{a}	176.4^{b}
TAKE-20	Male	4.57^{a}	205.9^{b}
	Female	4.21^{a}	157.8^{b}
BAYGON+ TAKE-20	Male	3.92	151.6^{b}
	Female	4.36^{a}	225.0^{a}

* Mean=SEM± 5 rats in each group.
a=P<0.05 b=P<0.01

A significant decrease in aminopyrine N-demethylase activity was observed during Baygon treatment. The percent decrease in the activity was same in both male and female rats. Take-20 injection resulted in an increase in aminopyrine N-demethylase activity. The percent increase was more in female rats as compared to the male rats. Baygon and Take-20 in combination also resulted in an increase of the enzyme activity. The percent increase was much more significant in male rats as compared to the female rats. PCMB at a concentration of 2.5×10^{-4} M inhibited aminopyrine N-demethylase activity and the percentage inhibition was 13.3 and 21.3 in male and female control group of rats, respectively. Due to the injection of Baygon the percentage inhibition due to PCMB was decreased in male rats and increased in female rats as compared to their control animals, however, the percentage inhibition during Take-20 intoxication was increased in male and decreased in female. The injection of Baygon and Take-20 in combination resulted in an increase in the percentage of inhibition of aminopyrine N-demethylase in presence of PCMB, irrespective of the sex of the animals.

Baygon intoxication resulted in a decrease in acetanilide hydroxylase activity both in male and female rats. The magnitude of decrease was more in male rats as compared to the female rats. The activity was increased by Take-20 injection and the percentage increase was more in male than in the female rats. When both the insecticides were injected in combination the activity was decreased. The decrease was much more significant in male as compared to the female.

TABLE 2 - Effect of insecticides and their combination on aminopyrine N-demethylase and acetanilide hydroxylase activities in young growing rats.*

Group	Sex	Aminopyrine N-demethylase activity. nM/min./mg. protein.			Acetanilide hydroxylase activity μM/min./mg protein.		
		-PCMB	+PCMB	% inhibition.	- PCMB	+ PCMB	% inhibition.
CONTROL	Male	15.0±0.3	13.0±0.1	-13.3	1.23±0.01	0.60±0.1	-51.2
	Female	7.5±0.1	5.9±0.2	-21.3	0.76±0.1	0.32±0.05	-57.9
BAYGON	Male	12.5±0.2^{b}	11.7±0.1	- 6.4	0.92±0.01^{b}	0.40±0.02	-56.5
	Female	6.25±0.2^{b}	4.4±0.2	-29.6	0.64±0.03^{b}	0.31±0.04	-51.5
TAKE 20	Male	16.3±0.2^{a}	12.3±0.1	-24.5	1.56±0.1^{b}	0.87±0.6	-42.7
	Female	8.75±0.2^{b}	7.5±0.2	-17.3	0.83±0.01^{a}	0.50±0.03	-38.3
BAYGON + TAKE 20	Male	18.3±0.2^{b}	11.0±0.2	-39.9	0.94±0.1^{b}	0.36±0.02	-61.7
	Female	8.12±0.2^{a}	4.6±0.2	-43.3	0.72±0.01	0.38±0.04	-47.2

* SEM = Mean ± 5 rats in each group.
a = $P < 0.05$
b = $P < 0.01$

In the control group of rats the percentage inhibition of acetanilide hydroxylase activity was more in female rats as compared to the male rats during *in vitro* addition of PCMB. The percent inhibition was increased in male rats when the two insecticides were injected in combination, however, the percent inhibition was decreased in the female rats, when Baygon, Take-20 or combination of the two insecticides were injected.

TABLE 3

Effect of insecticides and their combination on enzymatic and non-enzymatic lipid peroxidation in young growing rats*.

Group	Sex	Enzymatic lipid peroxidation**	Non-enzymatic lipid peroxidation**
CONTROL	Male	7.5±0.1	3.5±0.1
	Female	4.4±0.05	3.4±0.05
BAYGON	Male	7.7 ± 0.05^{a}	4.1 ± 0.1^{b}
	Female	4.6 ± 0.1^{a}	3.6 ± 0.1^{a}
TAKE-20	Male	9.1 ± 0.3^{b}	4.1 ± 0.1^{b}
	Female	4.9 ± 0.2^{b}	4.9 ± 0.2^{c}
BAYGON+ TAKE-20	Male	7.6±0.05	4.0 ± 0.2^{b}
	Female	3.9 ± 0.4^{a}	2.9 ± 0.1^{b}

* SEM=Mean ± 5 rats in each group.
** nM malonaldehyde formed/min/mg protein.
a=$P<0.05$ b=$P<0.01$ c=$P<0.001$

The enzymatic and non-enzymatic lipid peroxidations were increased during intoxication of insecticides (TABLE 3), however, when the two insecticides were given in combination lipid peroxidation was increased in male and decreased in female rats.

DISCUSSION

Many toxic insecticides by virtue of their inhibition of cholinesterase also inhibit other enzymes, but the toxicologic significance of this inhibition, while often suspected has not been established. It is well known that organophosphorus and carbamate insecticides serve as substrates for the hepatic microsomal enzymes(O'BRIEN 1967). HOFFMAN et al.(1970) have reported that acute treatment of insecticides result in an

increase in the activity of drug enzymes. We have also recently reported similar observations(MAKHIJA and PAWAR 1974). The increase in drug metabolizing enzyme activities at low concentration of Take-20 may be due to an increase in the chain length of the compound(DAUTERMAN and MAIN 1966). The increase in aminopyrine N-demethylase and decrease in acetanilide hydroxylase activity due to combined action of Baygon and Take-20 may possibly be due to the altered affinity of type I and type II sites of hemoproteins for respective substrates. The metabolism of insecticides proceed in three phases- the initial phase, the adaptation phase and the toxicity phase, where adaptation can no longer exist. The decrease in the activity of drug metabolizing enzymes during Baygon treatment in both male and female rats may be due to the toxic metabolites produced in the body. The compound injected may disrupt the association between the hemoprotein and the microsomal lipids. The primary or secondary action of the drug causes conformational changes in the hemoprotein which results in change in the activities of the enzymes. Obvious signs of salivation, fasciculations, etc., were observed immediately after insecticide intoxication.

PCMB acts by blocking the SH groups essential for the interaction between the flavoenzyme and cytochrome. It may also change the comformation or active sites of the enzymes to some extent so that inhibition of the enzyme activity takes place. It was observed that with the same concentration of PCMB the inhibition of N-demethylase and hydroxylase were more in females as compared to the males in the control group of rats. Sex variation could be explained on the basis of sex hormones, possibly due to the alterations in hormonal secretion.

The enzymatic and non enzymatic lipid peroxidations were increased due to insecticide intoxication. Our earlier reports indicate an increase in lipid peroxide formation during low dose of insecticide intoxication(MAKHIJA and PAWAR 1974,1975). UV spectra of total lipids have further indicated an increase in <u>in vivo</u> lipid peroxida formation band at 260-265 nm and the presence of diene conjugation band at 230-235 nm. Although it is considered likely that lipid peroxidation leads to membrane disintegration but the present studies indicate that the drug metabolism and lipid peroxidation proceed on two different pathways. Similar observations with the two systems have been also indicated by KAMATAKE and KITAGAWA (1974).

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