Effect of Insecticide Intoxication on the Hepatic Microsomal Electron Transport Reactions, During Dietary Protein Variations in Young Rats

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Induction of hepatic microsomal hydroxylative enzyme activities by drugs, pesticides and other foreign compounds is recognized as an important adaptive process in animals, a process markedly influencing the toxicity of such agents. WAGATAFF and STREET(1971) observed that ascorbic acid deficiency impaired the induction by dieldrin as early as two days on deficient diet. Rats fed on protein deficient diet or low protein diet(3.5-5% caesin) were more susceptible to malathion poisoning (BOYD and TANIKELLA 1969, BOYD et al. 1970, WEBB et al. 1973). Earlier we have demonstrated the effect of Baygon and Take-20 on drug metabolism and lipid peroxidation in young growing rats(1974,1975). However, reports pertaining to insecticide intoxication during dietary protein variation on hepatic drug metabolizing enzymes and lipid peroxidation are not available and hence the present studies were undertaken to investigate the effect of insecticides in relation to dietary protein intake on hepatic microsomal drug metabolizing enzymes and lipid peroxidation in young growing male rats.

MATERIALS AND METHODS

C.F.strain young male albino rats weighing initially 70-80 gms(obtained from M/S Ghosh and Co., Calcutta) were housed in individual cages and fed on a synthetic diet and water ad libitum at least for 15 days prior to the initiation of the experiments. The animals were then divided into the following three groups each containing 15 animals.

1) Control group of animals(18% caesin),
2) Low protein fed group of animals(5% caesin) and
3) high protein fed group of animals(30% caesin).

The animals were pair fed for two weeks. The composition of the diet was as reported previously(PATEL and PAWAR 1974) except that caesin was 18% in the case of control group of rats.

At the end of two weeks 5 animals from all three groups were injected intraperitoneally with Take-20(O, O-dimethyl malathion,150 mg/kg) in corn oil daily in the morning between 8.0-9.0 a.m. for two successive days. Another batch of 5 animals from all three groups were

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similarly treated with Baygon(10 mg/kg) in corn oil. The remaining animals served as control which received only corn oil.

The animals were sacrificed 24 hours after the last injection by decapitation. The liver was carefully perfused with 0.9% ice cold saline, excised, blotted dry, weighed, minced and homogenized(1:4 w/v) in ice cold 50 mM Tris-HCl buffer pH 7.4 containing 1.15% KCl using a Teflon pestle glass homogenizor. The microsomes were prepared as described by BAKER et al.(1973). The microsomal protein was estimated according to the biuret method(GORNALL et al.1949).

The drug enzyme assays were carried out as reported earlier(PATEL and PAWAR,1974). The method of NASH (1953) was used for the determination of aminopyrine and ethylmorphine N-demethylase activities. The p-hydroxyacetanilide formed during the hydroxylation of acetanilide was measured as described by WEISBURGER and GOODALL (1968).

The levels of microsomal electron transport components were estimated on a Hitachi(Model 124) recording spectrophotometer. The total heme content was determined by the pyridine hemochromogen procedure by observing the change in absorbance at 557-575 mμ. The heme content was calculated using 32.5 $cm^{-1}mM^{-1}$as the extinction coefficient. Cytochrome b5 and cytochrome c reductase were determined as reported earlier(PATEL and PAWAR,1974).

The NADPH linked and nonenzymatic lipid peroxidations were assayed as described by ERNSTER and NORDENBRAND(1967). The malonaldehyde formed was estimated by the thiobarbituric acid reaction(BERNHEIM et al.1948).

RESULTS

The relative liver weights were increased by insecticide intoxication(Table 1).The magnitude of increase was more in the animals fed on low protein diet as compared to those fed on high protein or normal diet. Due to the dietary protein variation, the insecticide treatment influenced the microsomal protein variably.

Baygon and Take-20 administration decreased the aminopyrine N-demethylase activity due to variation of dietary protein(Table 2). The magnitude of decrease was more in low protein injected animals as compared to high protein fed animals. Similarly, decrease in the activity of ethylmorphine N-demethylase was observed when low protein fed animals were intoxicated with Baygon, however, contrary to Baygon injection with Take-20 a noticeable increase in the activity of ethylmorphine N-demethylase was observed. Decrease in the ethylmorphine N-demethylase activity was also seen when both Baygon

TABLE 1

Effect of diet and insecticides on relative liver weights and microsomal protein contents

Group	Insecticide	Liver wt % body wt(gms)*	Microsomal protein mg/gm liver
CONTROL	None	3.48	55.0
	Baygon	3.75[a]	46.8[a]
	Take-20	3.95[a]	66.8[b]
LOW PROTEIN	None	3.60	42.7
	Baygon	3.96[a]	52.6[b]
	Take-20	4.23[b]	59.6[c]
HIGH PROTEIN	None	3.97	55.2
	Baygon	4.29[a]	62.5[a]
	Take-20	4.17[a]	61.3[a]

*SEM=Mean±5 rats in each group.
a=P<0.05 b=P<0.01 c=P<0.001

TABLE 2

Effect of diet and insecticides on drug metabolism

Group	Insecticide	Aminopyrine*	Ethylmorphine*	Acetanilide**
CONTROL	None	16.8±0.2	15.5±0.2	1.11±0.01
	Baygon	20.6±0.3[b]	19.5±0.2[a]	1.14±0.01
	Take-20	18.6±0.2[a]	17.2±0.1[a]	0.81±0.02[b]
LOW PROTEIN	None	13.1±0.3	10.8±0.1	0.96±0.01
	Baygon	11.9±0.1[a]	11.0±0.1[a]	0.99±0.01
	Take-20	10.0±0.1[b]	8.9±0.4[b]	0.96±0.01
HIGH PROTEIN	None	20.6±0.1	16.6±0.1	1.46±0.01
	Baygon	20.1±0.1[a]	15.5±0.2[a]	1.37±0.02[a]
	Take-20	16.5±0.3[b]	16.2±0.1[a]	1.34±0.01[a]

* nM formaldehyde formed/min/mg protein.
** μM p-hydroxyacetanilide formed/min/mg protein.
a=P<0.05 b=P<0.01

or Take-20 were injected to high protein fed animals.

Acetanilide hydroxylase activity was decreased when animals from high protein fed diet were intoxicated with Baygon and Take-20, whereas, it was increased when animals from low protein and control diet were injected with Baygon. As opposed to Baygon, Take-20 injection to

low protein fed group of rats did not influence any measurable change in enzyme activity.

A decrease in cytochrome b5, cytochrome c reductase and heme was noticed when rats from low and high protein were intoxicated with Baygon(Table 3), whereas,

TABLE 3

Effect of diet and insecticides on electron transport components

Group	Insecticide	Cytochrome b5 (nM/mg protein)	Heme	Cytochrome c reductase (nM/min/mg)
CONTROL	None	0.19	0.42	54.0
	Baygon	0.23[a]	0.50[a]	30.0[b]
	Take-20	0.17[a]	0.42	60.0[a]
LOW PROTEIN	None	0.13	0.46	18.0
	Baygon	0.10[a]	0.27[b]	12.0[b]
	Take-20	0.17[a]	0.46	12.0[b]
HIGH PROTEIN	None	0.19	0.60	36.0
	Baygon	0.15[b]	0.38[b]	30.0[b]
	Take-20	0.16[b]	0.46[b]	30.0[b]

a=P<0.05 b=P<0.01

in the case of control group of rats elevation in cytochrome b5 and total heme with a measurable decrease in cytochrome c reductase was noticed with the treatment of Baygon. Lower levels of cytochrome b5, cytochrome c reductase and heme were noticed when high protein fed animals were administered with Take-20. A decrease in cytochrome c reductase and increase in cytochrome b5 content was found when Take-20 was injected to low protein fed animals.

During insecticide intoxication a decrease in pyridine binding spectra was observed in low protein and high protein fed animals as compared to the animals from control group. The magnitude of decrease was more in low protein fed animals as compared to the high protein animals during Baygon intoxication(Figs 1,2,3).

The liver microsomal enzymatic and nonenzymatic lipid peroxidations were increased due to the treatment of insecticides, irrespective of the dietary variations, however, the magnitude of increase in lipid peroxidation was high with Baygon administration in high protein fed animals as compared to animals from low protein and control groups. The magnitude of increase in lipid peroxidation during Take-20 injections was more in low protein dietary state as compared to the other two states. (Table 4)

Fig 1.Effect of insecticides on pyridine binding Spectra in control diet fed animals.

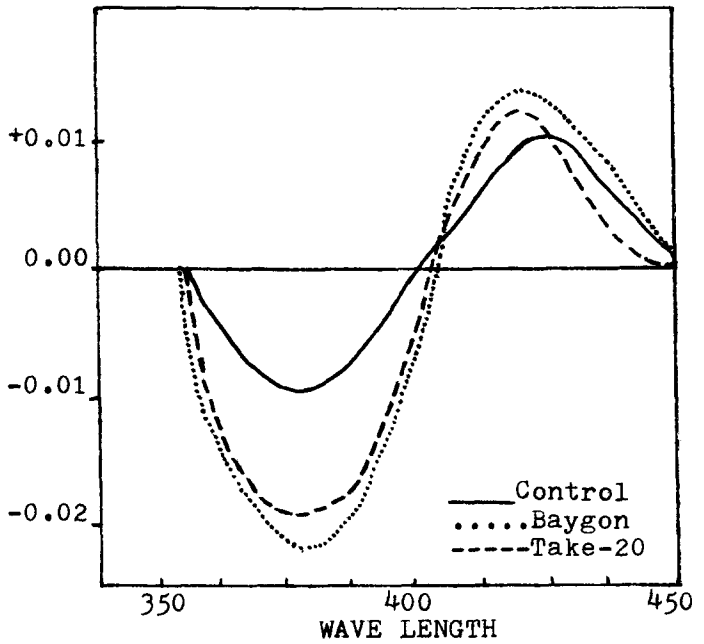

Fig 2.Effect of insecticides on pyridine binding Spectra in low protein fed animals.

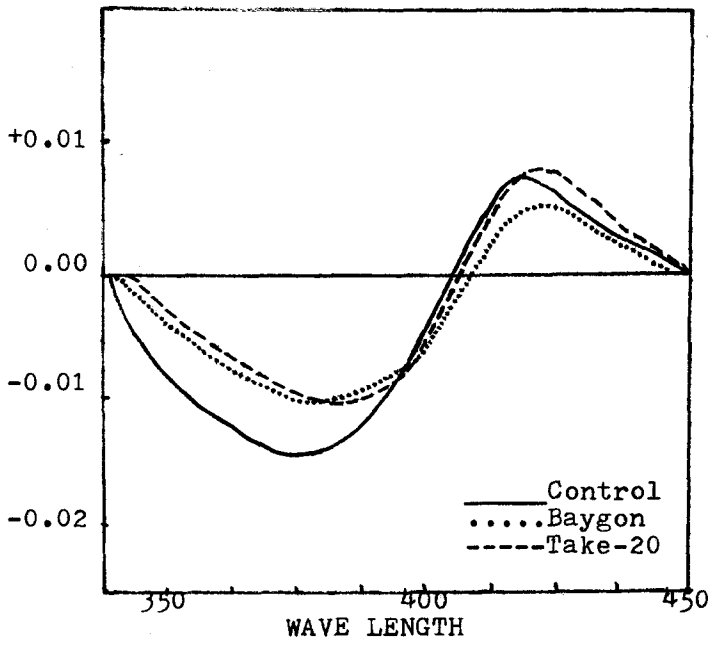

Fig-3.Effect of insecticides on pyridine binding spectra in high protein fed animals.

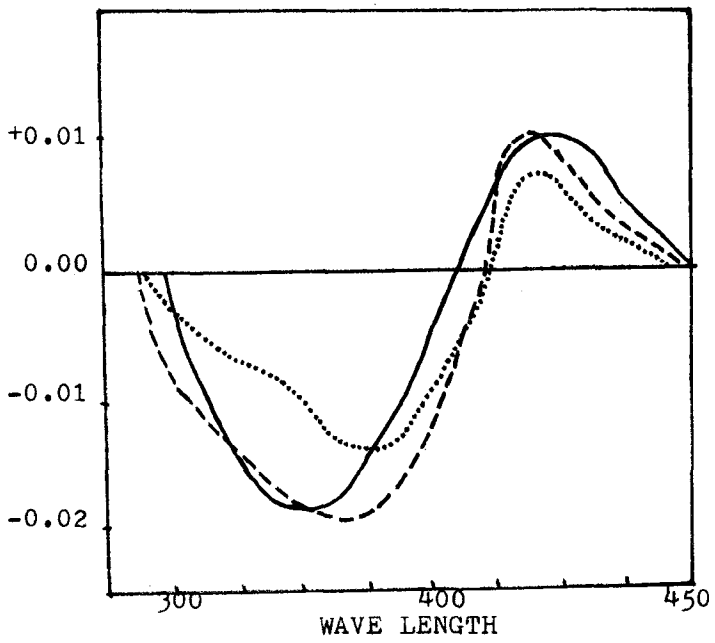

TABLE 4

Effect of diet and insecticides on lipid peroxidation

Group	Insecticide	Enzymatic (nM malonaldehyde formed/min/mg protein)	Nonenzymatic (nM malonaldehyde formed/min/mg protein)
CONTROL	None	8.6±0.2	4.0±0.2
	Baygon	11.1±0.3^{b}	6.0±0.3^{c}
	Take-20	9.9±0.2^{a}	6.7±0.2^{c}
LOW PROTEIN	None	9.0±0.1	5.1±0.1
	Baygon	9.8±0.2^{a}	5.3±0.1^{a}
	Take-20	17.8±0.3^{c}	8.6±0.2^{c}
HIGH PROTEIN	None	2.8±0.3	2.0±0.1
	Baygon	4.0±0.2^{c}	2.8±0.2^{c}
	Take-20	3.2±0.2^{a}	2.8±0.2^{c}
	a=P<0.05	b=P<0.01	c=P<0.001

DISCUSSION

The observed increase in relative liver weights in low protein fed animals due to insecticide intoxication could be due to glucose repression effect owing to increased carbohydrate intake besides protein deficiency during insecticide intoxication.

The variation in the drug enzyme activities due to dietary intake may be as a result of a deficiency of the apoenzyme or the change in the configuration of the membrane protein association with the normal oxidase function. The observed lower activity of drug enzymes in animals fed on high protein and low protein diet due to intoxication with Baygon and Take-20 could be due to decrease in the levels of cytochrome b5, cytochrome c reductase and heme content. The insecticide intoxication during dietary variations could induce certain conformational changes which would lead to facilitation of catalysis for the substrates. Alternatively, dietary protein insufficiency could lead to a more indirect effect either through an alteration in synthesis or breakdown of other components required for the normal catalysis by the enzyme system. It is also possible that dietary protein variation may change the hepatic detoxification of insecticides which would lead to increased toxicity. Low protein diets have been shown to protect the toxic effects of dimethynitrosamine(MCLEAN and MAGEE 1970, MCLEAN and VERSCHUREN 1969). However, the change in the levels of heme protein interactions during insecticide intoxication would be an additional factor during dietary protein stress. BOYD and DECASTRO(1968) concluded that low protein diets did not significantly alter DDT toxicity in the rat. Similar studies with lindane(BOYD and CHEN 1968) revealed that rats fed with cachectic diets were twice as susceptible to lindane poisoning as were control group of rats, however, WEATHERHOLTZ et al.(1969) reported that acute toxicity of heptachlor was affected 2-3 fold due to dietary protein levels. Recently, it has been reported that dietary composition and intake of calories affected induction of microsomal drug metabolizing enzymes(MARSHALL and MCLEAN 1971).

The observed increase in lipid peroxidation during insecticide intoxication confirms our earlier reports(MAKHIJA and PAWAR 1974,1975). Increased lipid peroxidation due to Baygon and Take-20 intoxication, and decreased drug metabolizing enzymes and microsomal electron components during variable dietary states, indicate the favourable membrane environment for the lipid peroxidation. Increased levels in cofactors and decrease in the membrane inhibitors due to the intoxication of insecticides under different dietary conditions could be an additional influencing factor. The observed slight different response due to insecticides during protein variation could be explained by the structural variation of the two insecticides.

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