Phenobarbital Induced Effect on Pulmonary and Hepatic Microsomal Ethylmorphine N-demethylase and Lipid Peroxidation during Oral Intoxication of Organic Solvents in Rats

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Several chemicals can induce drug metabolizing enzymes in hepatic microsomes (CONNEY 1967). The lung microsomal fraction also possesses mixed function oxidases capable of hydroxylating certain foreign compounds (UEHELKE 1968, OPPELT et al 1970). The information available regarding extrahepatic microsomal drug metabolism in rats is inadequate. So, investigations were undertaken to study the pulmonary and hepatic microsomal ethylmorphine N-demethylase and lipid peroxidation in adult male rats intoxicated with organic solvents, benzene and toluene.

MATERIALS and METHODS

The Hindustan antibiotic strain adult male rats (200-250 gms) were obtained from Hindustan Antibiotics Poona. All animals were fed on a synthetic diet one week before initiating the experiments. The composition of the diet was as reported earlier (PATEL and PAWAR 1974) except that the casein content was 18%. The rats were then divided into the following groups- 1) Control, 2) Benzene treated 3) Toluene treated 4) Phenobarbital treated 5) Phenobarbital and benzene treated 6) Phenobarbital and toluene treated. During the entire experimental period the animals were pair fed.

Benzene(0.80 ml/kg) and toluene(0.72 ml/kg) were administered orally to the rats daily in the morning for two successive days. The animals from group 4,5 and 6 were injected with phenobarbital sodium (80 mg/kg) for three days. The phenobarbital treated animals from group 5 and 6 were further treated with benzene(0.80 ml/kg) and toluene(0.72 ml/kg) respectively for two days.

The animals were sacrificed 24 hours after the last treatment by decapitation. The lungs and livers were removed as rapidly as possible after perfusion with 0.9% ice cold saline. The lungs were blotted dry seperated from the trachea and major bronchi and homogenized(1:6 w/v) in ice cold 50 mM Tris-HCl buffer pH 7.4 containing 1.15% KCl for 40 sec. at 5000 rpm with an Osterizer blendor (Model Imperial VIII)with a mini container. The livers were similarly homogenized (1:4 w/v) in ice cold 50 mM Tris-HCl buffer containing 1.15% KCl with a teflon pestle glass homogenizer. The microsomes were isolated by the $Ca^{2+}Mg^{2+}$ sedimentation procedure of BAKER et al,(1973) using a refrigerated centrifuge (Remi K-24). The microsomal protein was estimated by the biuret method (GORNALL et al 1949) using crystalline bovine serum albumin as the standard.

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The ethylmorphine N-demethylase assay was carried out in a medium containing 50 mM Tris-HCl buffer pH 7.4, 5 mM $MgCl_2$, 8 mM ethylmorphine, 0.2 mg NADPH and 2 mg hepatic microsomal protein /ml and 4 mg lung microsomal protein/ml. The reactions were initiated by the addition of NADPH. All incubations were carried out in a Dubnoff metabolic shaker at 37^0c. The formaldehyde formed during N-demethylation was estimated by the method of NASH(1953) The NADPH linked lipid peroxidation was assayed as described by ERNSTER and NORDENBRAND(1967). The ascorbate induced lipid peroxidation was assayed in the same medium as NADPH linked except that NADPH was omitted and nicotinamide was replaced by 1 mM ascorbate. The thiobarbituric acid reaction was used for the estimation of malonaldehyde formation (BERNHEIM et al,1948).

The lung and liver microsomal total lipids were extracted according to the Folch's method(FOLCH et al 1957) The UV spectra of liver and lung microsomal lipids were carried out to examine the presence of diene conjugation band and malonaldehyde formation.

RESULTS

The relative lung weights were not affected due to benzene and toluene intoxication, however, the relative liver weights were slightly increased due to benzene intoxication. The lung microsomal protein was decreased due to organic solvents. The magnitude of decrease was much more significant with toluene as compared to benzene administration. However, an increase in the liver microsomal protein was observed due to the treatment of benzene and toluene. The degree of increase in liver microsomal protein was higher with toluene intoxication as compared to benzene.

Phenobarbital pretreatment could not significantly alter relative lung or liver weights during benzene intoxication, whereas, it could increase the relative lung or liver weights during toluene intoxication. Phenobarbital pretreatment did not have any effect on the lung microsomal protein during intoxication with organic solvents, whereas, it had inductive effect on liver microsomal protein in benzene and toluene intoxicated animals, The magnitude of induction due to phenobarbital was higher in toluene treated animals as compared to benzene treated animals.

Both the hepatic and pulmonary ethylmorphine N-demethylase activities were affected due to benzene and toluene intoxication and the magnitude of decrease was variable in both the organs (Table 2)

Phenobarbital pretreatment could not induce and protect ethylmorphine N-demethylase activity, whereas, a dissimilar pattern was observed in liver during toluene intoxication. However, during benzene administration the

effect was similar in lung and liver,

TABLE 1

Effect of organic solvents on relative organ weight and organ protein content with and without pretreatment of phenobarbital in adult male rats*

Group	Relative lung wt. (gms)	Relative liver wt. (gms)	Lung microsomal protein (mg/gm lung)	Liver microsomal protein (mg/gm liver)
Control	0.69	3.5±0.1	27.2±0.2	51.0±1.0
Benzene treated	0.71	3.9±0.5[a]	25.7±0.6[a]	54.0±1.0[a]
Toluene treated	0.69	3.9±0.2[a]	23.5±1.2[b]	60.5±0.5[a]
Phenobarbital treated	0.74	4.1±0.2	23.2±0.5	71.0±1.0
Phenobarbital + benzene treated	0.63[a]	4.1±0.2	17.6±0.8[a]	94.5±0.5[b]
Phenobarbital + toluene treated	1.02[a]	4.8±0.4[a]	15.8±2.0[b]	109.0±1.0[b]

* = Mean ± SEM (6 rats in each group).
a = $P < 0.01$ b= $P < 0.001$

Both the NADPH and ascorbate induced lipid peroxidation was decreased in both lung and liver due to benzene intoxication. The registered level of lung lipid peroxidation increased due to toluene intoxication, whereas, liver lipid peroxidation was noticeably decreased.

Phenobarbital pretreatment decreased the lung lipid peroxidation during benzene intoxication, however it could not exhibit the same influence during toluene intoxication. The injection of phenobarbital prior to benzene and toluene intoxication resulted in a decrease in liver lipid-peroxidation. The magnitude of decrease was more in toluene treated animals as compared to benzene treated ones.

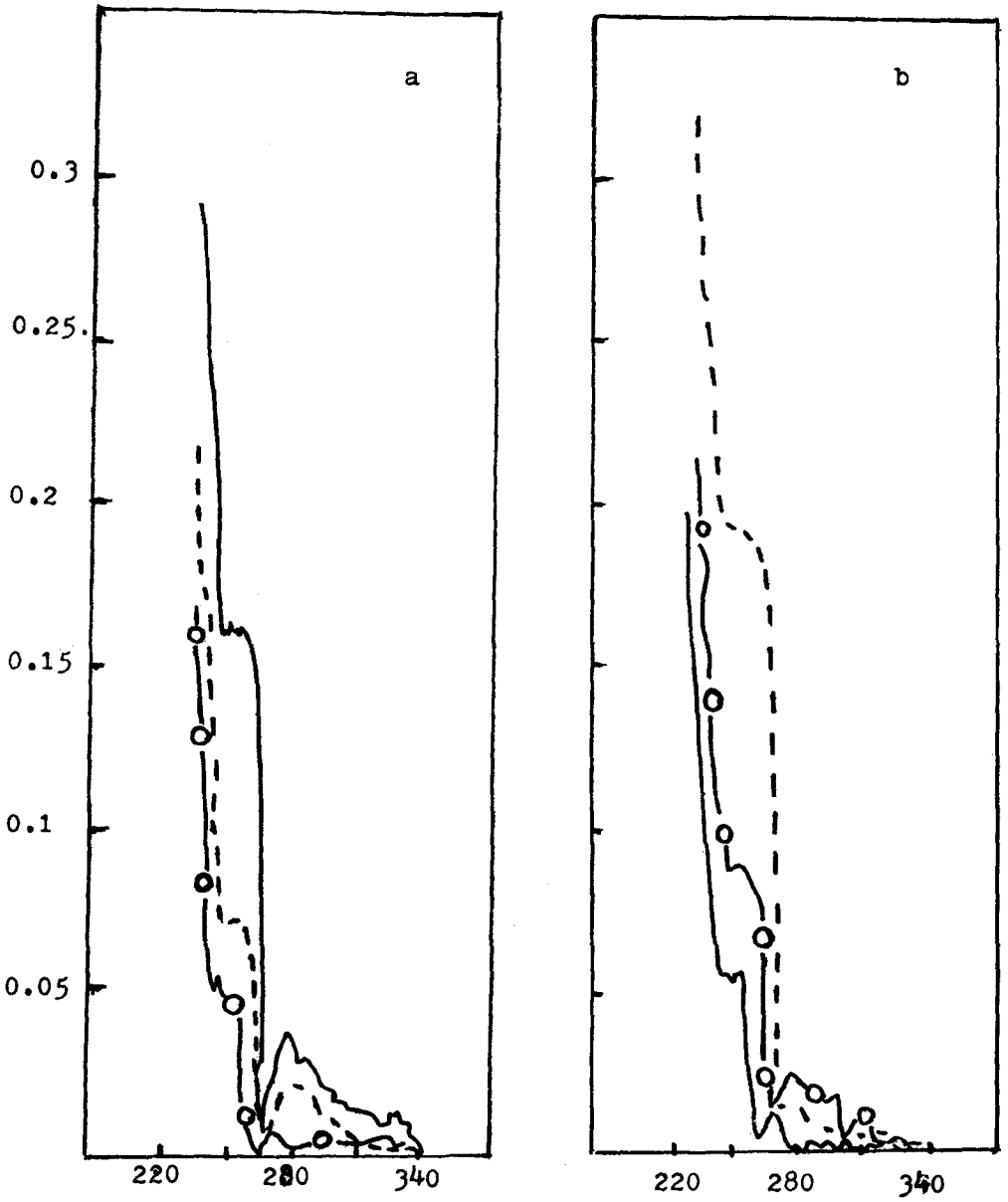
TABLE 2. Effect of organic solvents on hepatic and pulmonary N-demethylase, lipid peroxidation and lipid content with and without pretreatment of phenobarbital in adult male rats*.

Group	Ethylmorphine N-demethylase**		NADPH linked lipid peroxidation**		Ascorbate induced ** lipid peroxidation		Total microsomal lipids***	
	Lung	Liver	Lung	Liver	Lung	Liver	Lung	Liver
Control	1.56±0.2	25.7±1.7	2.5±0.1	16.2±0.2	4.4±0.1	17.8±0.2	0.46	1.87
Benzene treated	0.94±0.3b	15.5±0.5b	2.2±0.1a	14.6±0.2a	4.1±0.2a	15.8±0.2a	0.84b	0.45b
Toluene treated	0.78±0.1b	19.0±1.0b	2.7±0.1a	13.8±0.4b	5.2±0.2b	13.9±0.8a	0.69b	0.71b
Phenobarbital treated	2.25±0.2	42.0±2.0	-	14.0±0.4	3.5±0.3	14.8±0.4	1.67	1.34
Phenobarbital + benzene treated	2.00±0.1a	38.7±1.2a	-	11.8±0.2a	2.7±0.3a	14.4±0.6	1.97a	0.40b
Phenobarbital + toluene treated	1.55±0.1b	27.0±3.0b	-	13.0±0.2a	5.3±0.4b	12.7±0.2a	2.70b	0.29b

* Mean ± SEM (6 rats in each group)
** nM product formed/min/mg protein.
*** mg/mg protein.

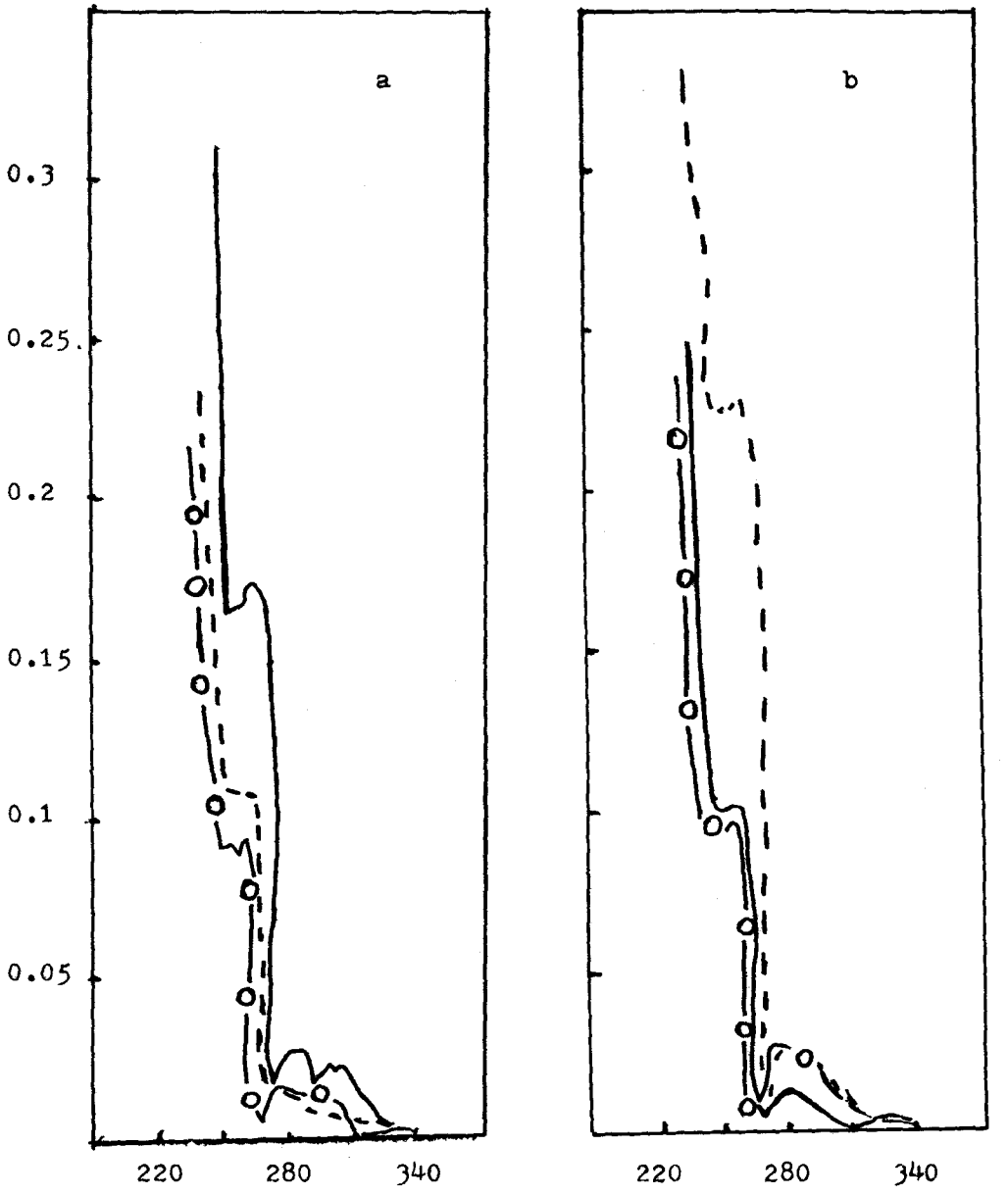
a= P 0.01
b= P 0.001

.Fig. 1: UV spectrum of liver microsomal lipids in adult male rats. (a) ——— Control animals; -0-0- benzene treated animals; ----- toluene treated animals. (b) ——— Phenobarbital treated animals; -0-0- phenobarbital and benzene treated animals; ---- phenobarbital and toluene treated animals.



Abscissa: wavelength(mμ)
Ordinate: absorbance.

Fig 2: UV spectrum of lung microsomal lipids in adult male rats. (a) ——— Control animals; -0-0- benzene treated animals; ----- toluene treated animals. (b) ——— Phenobarbital treated animals; -0-0- phenobarbital and benzene treated animals; ----- phenobarbital and toluene treated animals.



Abscissa: wavelength(mμ)
Ordinate: absorbance.

The lung microsomal total lipids were increased due to benzene and toluene administration, however, the liver microsomal total lipids were decreased. The UV spectrum of lung and liver microsomal lipids exhibited a decrease in diene conjugation band and malonaldehyde formation due to the administration of benzene and toluene.

The injection of phenobarbital prior to the intoxication of organic solvents increased the lung microsomal total lipids. The magnitude of increase was higher in the case of toluene treated animals as compared to benzene treated animals. Phenobarbital pretreatment resulted in an increase in total lipids due to benzene intoxication however, the total lipids were decreased when toluene was administered to induced rats, whereas, the diene conjugation band did not change during benzene intoxication to phenobarbital induced rats. In liver microsomal lipids the diene conjugation registered an increase during the administration of benzene and toluene to phenobarbital pretreated rats.

DISCUSSION

In the present studies the pulmonary microsomal ethylmorphine N-demethylase activity was found to be 5% of the hepatic microsomal activity in adult male rats. KLINGER (1973) has reported amidopyrine N-demethylase activity in the lung to be 2% of the liver activity in adult rats using 9000xg as an enzyme source. Several workers have described low levels of oxidative metabolism in rat lung (OPPELT et al 1970, GILMAN and COONEY 1963 GILBOIN and BLACKBURN 1964, WELCH et al 1971). This discrepancy between the change could be due to the change in the enzyme source.

The decrease in the drug metabolizing enzyme activities of the liver due to treatment of organic solvents could be due to the reduced levels of heme, cytochrome c reductase, cytochrome P450 and/or the loss of structural integrity and the change in the environment of the microsomal enzyme system. The protective effect of phenobarbital on pulmonary and hepatic ethylmorphine N-demethylase activity during benzene and toluene intoxication could be due to the induced drug metabolizing enzymes which could enhance the *in vivo* metabolism of benzene and toluene.

IKEDA and OHTSUJI(1971) reported that phenobarbital pretreatment increased the hepatic aromatic hydroxylase and *in vivo* metabolism of benzene and toluene in rats. GONASUN et al (1973) reported an increase in *in vitro* benzene metabolism without increasing cytochrome P450 during benzene treatment in mice. SAITO et al (1973) observed an increase in the metabolism of zoxazolamine, neoprontosil and p-aminobenzoic acid but not of hexobarbital when benzene was given in a single large dose to rats. Recently DREW and FOUTS(1974) have shown that pheno-

barbital and 3-methylcholanthrene induced benzene metabolism in the liver, but did not affect the acute inhalation toxicity or the ip toxicity of benzene. The observed variations in the present studies could be due to variable factors such as different species, strain sex and age of the experimental animals. The route of organic solvent administration might be an additional factor.

The low levels of lipid peroxidation during benzene intoxication might be possibly due to the metabolites (epoxides) of benzene formed acting as antioxidants in the lung and liver. It is possible that the protein lipid interactions are affected due tothe organic solvents. The metabolites of toluene might be inhibiting the *in vivo* lipid peroxidation. The slight change in the diene conjugation band in the lung may be due to organ variation response for *in vivo* lipid peroxidation. The reduced levels of ascorbate could not be ruled out as a contributory dynamic; however, the presence of *in vitro* ascorbate could be an additional influencing factor. The decrease in lipid peroxidation due to phenobarbital pretreatment during benzene intoxication could be due to the competition for a common flavoprotein cytochrome c reductase.

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