Hepatic Microsomal Mixed Function Oxidase System during Toluene Treatment and the Effect of Pretreatment of Phenobarbital in Adult Rats

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Many studies during the last few years have shown that certain chemicals by their stimulation or inhibition of microsomal enzymes may alter the toxicity of other chemicals to which man is exposed. Because of the almost inevitable exposure of urban dwellers to numerous noxious chemicals, biochemical systems metabolizing these chemicals is of importance. IKEDA and OHTSUJI (1971) have observed that the treatment of rats with phenobarbital stimulated in vivo metabolism of toluene and benzene. More recently, DREW and FOUTS (1974) have shown that phenobarbital and 3-methylcholanthrene do induce in vivo metabolism of benzene in liver.

Attempts were made in the present investigations to study the effect of acute oral poisoning of toluene on the levels of hepatic microsomal drug metabolizing enzymes, cytochrome b_5, cytochrome c reductase and heme in in both male and female rats. The effect of phenobarbital pretreatment on toluene intoxication was also studied.

MATERIALS and METHODS

Hindustan antibiotic strain adult male (200-250 gms) and female (135-160 gms) albino rats were obtained from Hindustan-Antibiotics, Poona, India. The animals were kept in an air-conditioned room and supplied with rat pellets (obtained from Hindustan Lever Ltd., Bombay) ad libitum prior to the initiation of the experiments. All animals were fed on a synthetic diet for 1 week before initiation of the experiments. The composition of the diet was as reported earlier (PATEL and PAWAR)1974) except the casein content was increased to 18 percent. The rats were then alloted to the following groups and pair fed during the experimental period.

1) Control group of rats.
2) Toluene treated group of rats.
3) Phenobarbital treated group of rats.
4) Phenobarbital and toluene treated group of rats.

Toluene (0.72 ml/kg body weight) was administered orally to the rats using corn oil as a carrier in the morning before feeding for 2 successive days.

Phenobarbital sodium (80 mg/kg body weight) was injected intraperitoneally daily in the morning between 8.0-9.0 A.M. on each day for 3 days. Rats from group 4 which were treated with phenobarbital were further treated with toluene for 2 successive days.

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 2 © 1976 by Springer-Verlag New York Inc.

The animals were killed by decapitation, 24 hours after the last injection. The whole livers were carefully perfused with 0.9 % ice cold saline, excised, weighed minced and homogenized (1:4 w/v) in ice cold 50 mM Tris-HCl buffer, pH 7.4 containing 1.15 % Kcl. All tissue preparations were made at -2°c. The microsomes were isolated by the procedure of BAKER et al (1973), The microsomal protein was determined by the biuret method (GORNALL et al 1949) using crystalline bovine serum albumin as the standard.

Drug enzyme activities were carried out as reported earlier (PATEL and PAWAR 1974) using 1 mg/ml microsomal protein. Aminopyrine and ethylmorphine N-dealkylation reactions were measured by formaldehyde production by the Nash reaction (NASH 1953). Acetanilide hydroxylation was estimated by measuring the formation of p-hydroxyacetanilide (WEISBURGER and GOODALL 1968).

Microsomal NADPH cytochrome c reductase and cytochrome b_5 content were determined as reported recently (PATEL and PAWAR 1974). Total heme content was determined from the increments of absorbance between 557 and 575 mμ using alkaline hemochromogen procedure.

RESULTS.

TABLE 1

Changes in relative liver weights and liver microsomal protein in male and female rats due to the treatment of toluene and pretreated with phenobarbital.

Parameter	Control	Toluene treated	Phenobarbital treated	Phenobarbital + toluene treated.
Male				
Liver wt % body wt (gms)	3.5±0.1*	3.9±0.2^{c}	4.1±0.2^{b}	4.8±0.4^{b}
Microsomal protein mg/gm liver	51.0±1.0	60.5±0.5^{b}	71.0±1.0^{a}	109.0±1.0^{a}
Female				
Liver wt % body wt (gms)	4.2±0.2	4.3±0.1^{c}	4.6±0.3^{c}	4.4±0.3^{c}
Microsomal prptein mg/gm liver	58.0±1.0	65.0±1.0^{c}	97.5±2.5^{a}	111.0±1.0^{a}

* SEM = Mean±10 rats in each group.
a = P<0.05 b = P<0.01 c = P<0.001

Table 1 presents changes in liver weights and liver microsomal protein content. Toluene treatment did not show any significant change in relative liver weights. Administration of toluene resulted in an increase in microsomal protein. The percentage increase was 20 and 12 in male and female rats respectively.

Table 2 gives the activities of drug enzymes during various treatments. In agreement with various reports phenobarbital treatment resulted in marked increase in the activities of aminopyrine and ethylmorphine N-demethylases, whereas a very slight increase in the activity of acetanilide hydroxylase was observed due to phenobarbital treatment. The induction was more in male as compared to female animals.

TABLE 2

Effect of treatment of toluene on liver microsomal drug-metabolizing enzymes with and without pretreatment of phenobarbital in adult male and female rats.

Parameter	Control	Toluene	Phenobarbital treated	Phenobarbital+ toluene treated
Male				
Aminopyrine N-demethylase**	23.50±1.50*	14.25±1.75[a]	49.00±1.00[a]	36.25±1.25[a]
Ethylmorphine N-demethylase	25.75±1.75	19.00±1.00[b]	42.00±2.00[a]	27.00±3.00[a]
Acetanilide hydroxylase***	0.60±0.03	0.82±0.03[a]	0.64±0.10[c]	0.61±0.05[a]
Female				
Aminopyrine N-demethylase	21.25±1.25	19.35±0.65[c]	26.25±1.25[b]	23.75±1.25[b]
Ethylmorphine N-demethylase	16.25±1.25	12.50±2.50[b]	17.50±2.50[c]	17.50±2.50[a]
Acetanilide hydroxylase	0.43±0.05	0.57±0.03[a]	0.61±0.06[a]	0.58±0.03[c]

* SEM = Mean±10 rats in each group.
** = Activity express as nmoles of formaldehyde formed per min. per mg. protein.
*** = Activity express as µmoles of p-hydroxyacetanilide formed per min. per mg. protein.
a = $P < 0.05$ b = $P < 0.01$ c = $P < 0.001$

Toluene treatment resulted in a decrease in N-demethylations of aminopyrine and ethylmorphine. On the other hand a very slight increase in acetanilide hydroxylase was noticed in both male and female rats.

Rats pretreated with phenobarbital prior to the administration of toluene showed significantly higher activities of aminopyrine and ethylmorphine N-demethylases in male rats. On the contrary, phenobarbital pretreatment resulted in a decrease in the acetanilide hydroxylase activity in male rats; whereas, no effect was observed in female rats.

Table 3 shows the levels of microsomal electron transport components during various treatments. Phenobarbital treatment did not have any significant effect on the levels of cytochrome b_5 in male and female rats; however, total heme content was increased irrespective of sex. Cytochrome c reductase was increased due to phenobarbital treatment in male and female rats.

TABLE 3

Effect of treatment of toluene on the levels of hepatic microsomal cytochrome b_5, cytochrome c reductase and total heme with and without pretreatment of phenobarbital in adult male and female rats.

Parameter	Control	Toluene treated	Phenobarbital treated	Phenobarbital + toluene treated
Male				
Cytochrome b_5 nmoles/min/mg	0.14*	0.13[c]	0.14	0.11[b]
Cytochrome c reductase nmoles/min/mg	24.0	18.0[b]	30.0[b]	21.0[b]
Total heme nmoles/mg	0.38	0.31[b]	0.46[b]	0.53[a]
Female				
Cytochrome b_5 nmoles/min/mg	0.13	0.10[b]	0.18[a]	0.10
Cytochrome c reductase nmoles/min/mg	18.0	12.0[a]	24.0[a]	14.0[b]
Total heme nmoles/mg	0.31	0.23[b]	0.46[a]	0.61[a]

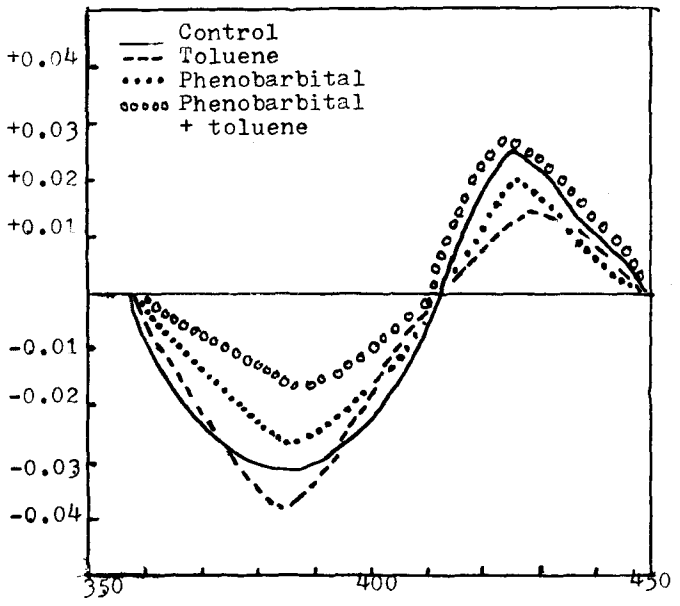
* Values are the average of three determinations of pooled livers of 3 rats. a = $P<0.05$, b = $P<0.01$, c = $P<0.001$.

Cytochrome b_5, cytochrome c reductase and total heme contents were lowered in animals treated with toluene. The decrease was slightly more in female rats as compared to male rats.

Phenobarbital pretreatment prior to the administration of toluene did show a slight protective effect on the activity of cytochrome c reductase although cytochrome c reductase level was lower than in rats treated with phenobarbital alone. The level of total heme also was significantly high due to phenobarbital pretreatment in toluene intoxicated rats

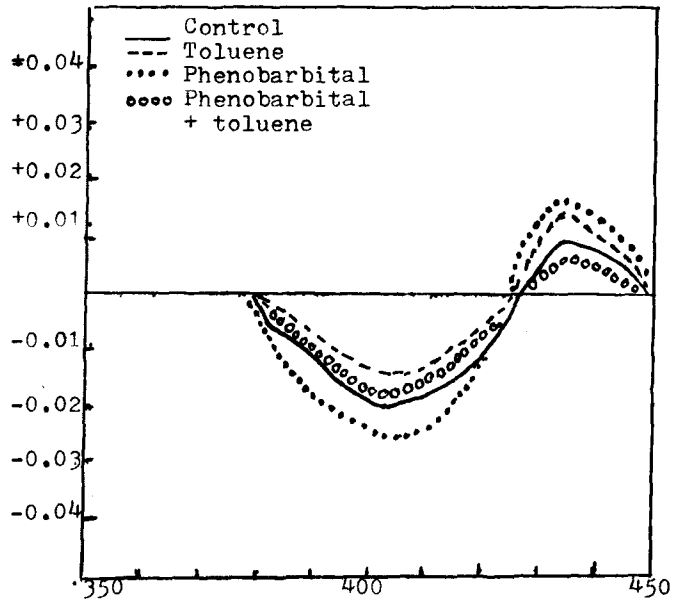
Effect of toluene treatment on pyridine binding spectra with and without pretreatment of phenobarbital in adult male (Fig.1) and female (Fig.2) rats.

Fig.1



WAVE LENGTH

Fig.2



WAVE LENGTH

A decrease in pyridine binding spectra was observed in toluene treated animals. The magnitude of pyridine binding spectra was also changed due to phenobarbital pretreatment prior to the administration of toluene (Figs 1 and 2)

DISCUSSION

The induced effect of phenobarbital treatment on liver microsomal mixed function oxidase system is well known. In selecting phenobarbital for pretreatment studies in toluene intoxication we have chosen this compound which induce two different interconvertible hemoproteins which is believed to be the terminal electron acceptor in drug hydroxylation reactions. The pretreatment of rats with phenobarbital could alter the toluene poisoning.

The present studies indicate an example of increase tolerance to pharmacological action of drugs. The results appear to suggest the possibility of using inducing agents of drug metabolizing enzymes to intoxication therapy due to organic solvents.

Decreased metabolism of aminopyrine and ethylmorphine in toluene treated rats could be explained by decreased levels of cytochrome c reductase, heme and/or the loss of structural integrity of microsomal system could be an additional factor. The observed sex variation in drug enzymes due to toluene treatment could be due to hormonal variation (KATO et al 1969, PATEL and PAWAR 1973). A slightly induced effect of toluene on acetanilide hydroxylase activity in both male and female rats indicate slightly enhanced substrate affinity for site II. The increase in microsomal protein content in toluene treated animals which was further induced due to the pretreatment of phenobarbital remains to be answered ; however, it is quite likely that catabolism might have inhibited.

Phenobarbital pretreatment protected the activities of ethylmorphine and aminopyrine N-demethylases from toluene intoxication. This could be due to the induced effect of phenobarbital on drug enzymes which could have resulted in enhanced *in vivo* metabolism of toluene.

IKEDA and OHTSUJI (1971) reported that phenobarbital pretreatment resulted in an enhanced *in vivo* metabolism of toluene, benzene and *in vitro* stimulation of aromatic hydroxylation in young rats and guinea pigs. DREW and FOUTS (1974) did not observe any protective effect of phenobarbital in female rats. They have shown that phenobarbital and 3-methylcholanthrene do induce *in vivo* benzene metabolism. GONASUN et al (1973) noticed the increase in *in vitro* benzene metabolism without increasing cytochrome P-450 content in benzene treated mice. They have also observed that the phenobarbital treatment resulted in an increase in cytochrome P-450 content without any stimulation of benzene metabolism. The variable protective effect due to phenobarbital pretreatment could be due to the variations in age, sex, strain, species of the animals and the route of administration of organic solvents.

In conclusion, the results indicate that toluene administration decreased the levels of aminopyrine and ethylmorphine N-demethylases. Phenobarbital pretreatment protects the levels of drug enzymes against the toluene intoxication. Levels of hepatic microsomal cytochrome b_5, total heme and cytochrome c reductase were lowered due to the administration of toluene in both male and female rats. The results suggest that toluene acts on a molecular level.

ACKNOWLEDGEMENTS

AMM is a Junior Research Fellow of University Grants Commission., New Delhi. SSP is grateful to Prof. Ronald W. Estabrook, Professor and Chairman, Department of Biochemistry, University of Texas, Health Science Center Dallas, Texas, USA for his generous help and encouragement. The authors wish to thank Dr. J.M.Patel (presently at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina USA) for his help during the early stages of the work.

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