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Effects of Dietary Lead Acetate on Hepatic Detoxication Enzyme Activity

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Lead-containing compounds usually inhibit enzymic and metabolic processes. This inhibition is presumed to be the mechanism of intoxication by these compounds. Inhibition of detoxication activities of liver microsomal enzymes could be particularly detrimental because the toxicity of many different substances would be increased. Exposure of experimental animals to lead compounds in several studies has been associated with depressed activity of hepatic microsomal enzymes, reduced levels of hepatic cytochrome P-450, reduced levels of hepatic microsomal protein, and prolonged hexobarbital sleep times (ALVARES et al. 1972, 1976; CHOW & CORNISH 1978, CRESS & LARSON 1970, GOLDBERG et al. 1978, SCOPPA et al. 1973, RIBEIRO 1971). The abundance of smooth endoplasmic reticulum in liver cells of rats was reduced by oral administration of lead acetate (HILDERBRAND et al. 1973).

In vitro addition of lead compounds to hepatic microsomal preparations inhibited enzyme activity in most studies (CHOW & CORNISH 1978, FOUTS & POHL 1971). CHOW & CORNISH (1978) demonstrated that inhibition probably would have occurred in all in vitro experiments if non-complex-forming buffer systems were used. Impairment of drug metabolism has been reported for some children and, to a lesser degree, adults exposed to lead (ALVARES et al. 1975, 1976; CAMPBELL et al. 1976, FISCHBEIN et al. 1977). On the other hand, there are some reports of lack of any enzymic inhibitory effect of lead (ALVARES et al. 1976, PHILLIPS et al. 1971). Benzpyrene-induced reduction of zoxazolamine paralysis time was not affected by lead (CHOW & CORNISH 1978). Also, after 2 or more days lead had no effect on the induction of cytochrome P-450 by phenobarbital (CHOW & CORNISH 1978, NORPOTH et al. 1974). EGAN & CORNISH (1973) observed initial depression of hepatic cytochrome P-450 in rats treated once with lead acetate but this was followed in 10-14 days by a doubling of cytochrome P-450 levels. The data were not sufficient to determine whether this delayed increase was merely a compensatory response after a period of depression or was due to some enzymic stimulatory effect associated with lead exposure. The present report contains observations that under certain experimental conditions there is stimulated hepatic microsomal enzyme activity in rats fed lead acetate.

METHOD

Female Holtzman rats (5 rats per group) which weighed 100-110 g at the start of each experiment were individually housed in suspended stainless steel wire-mesh cages. Glass feed jars and

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water bottles with stainless steel sipper tubes were used. The rats were fed a semipurified basal diet, i.e., USP vitamin A test diet fortified with 0.69 ppm vitamin A acetate.

In vivo detoxication enzyme activity was determined by the hexobarbital sleep time procedure as follows. After 10 days of feeding, each rat was injected intraperitoneally with 90 mg hexobarbital sodium/kg body weight, and the time period was recorded during which the righting reflex was absent. Decreased sleep time was accepted as presumptive evidence of stimulation of microsomal enzyme activity (AXELROD 1965).

In vitro determinations of enzyme activity were performed after 15 days' feeding. Each rat was killed with ethyl ether, the liver was excised, and a 1 g portion ground in a glass-Teflon homogenizer with 4 ml cold 1.15% KCl. The homogenate was centrifuged at 5°C for 15 min at 9000 X g. Detoxication enzyme activity in the supernatant fluid was determined by assaying oxidative cleavage of the insecticide O-ethyl O-(p-nitrophenyl) phenylphosphonothioate (EPN detoxication) and oxidative O-demethylation of p-nitroanisole (O-demethylase) according to the methods of KINOSHITA et al. (1966). The product of both reactions was p-nitrophenol (PNP). The remainder of each liver was frozen in an insulated chest containing solid CO_2 and was later transferred to a freezer. Microsomal cytochrome P-450 and microsomal protein were determined in the stored liver samples. Microsomal pellets were obtained by centrifuging the 9000 X g supernatant fluid at 100,000 X g for 1 h. Cytochrome P-450 content of the pellet was determined by the method of KLINGENBERG (1958). Microsomal protein was measured by the procedures of LOWRY et al. (1951).

In the first of the two experiments reported here, rats were fed diets fortified with 0, 100, 500, 1000, 5000, or 10,000 ppm reagent grade neutral lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$. The second experiment studied the interactions of feeding diets fortified with 1000 ppm lead acetate and 500 ppm USP grade phenobarbital sodium. The design was completely randomized with factorially arranged treatments. Four groups of rats were fed diets fortified with 1) no lead acetate and no phenobarbital, 2) 1000 ppm lead acetate and no phenobarbital, 3) no lead acetate and 500 ppm phenobarbital, and 4) 1000 ppm lead acetate and 500 ppm phenobarbital.

RESULTS

The effects of feeding graded dietary levels of lead acetate on hepatic microsomal enzyme activities are summarized in Fig. 1. Enzyme activities were not depressed at any exposure level of lead acetate even though body growth rates were markedly impaired in rats fed the three highest levels. In fact, there was a moderate level of enzyme stimulation, as indicated by increased activity of EPN detoxication and O-demethylase and decreased hexobarbital sleep time in rats fed 1000 ppm lead acetate. EPN detoxication

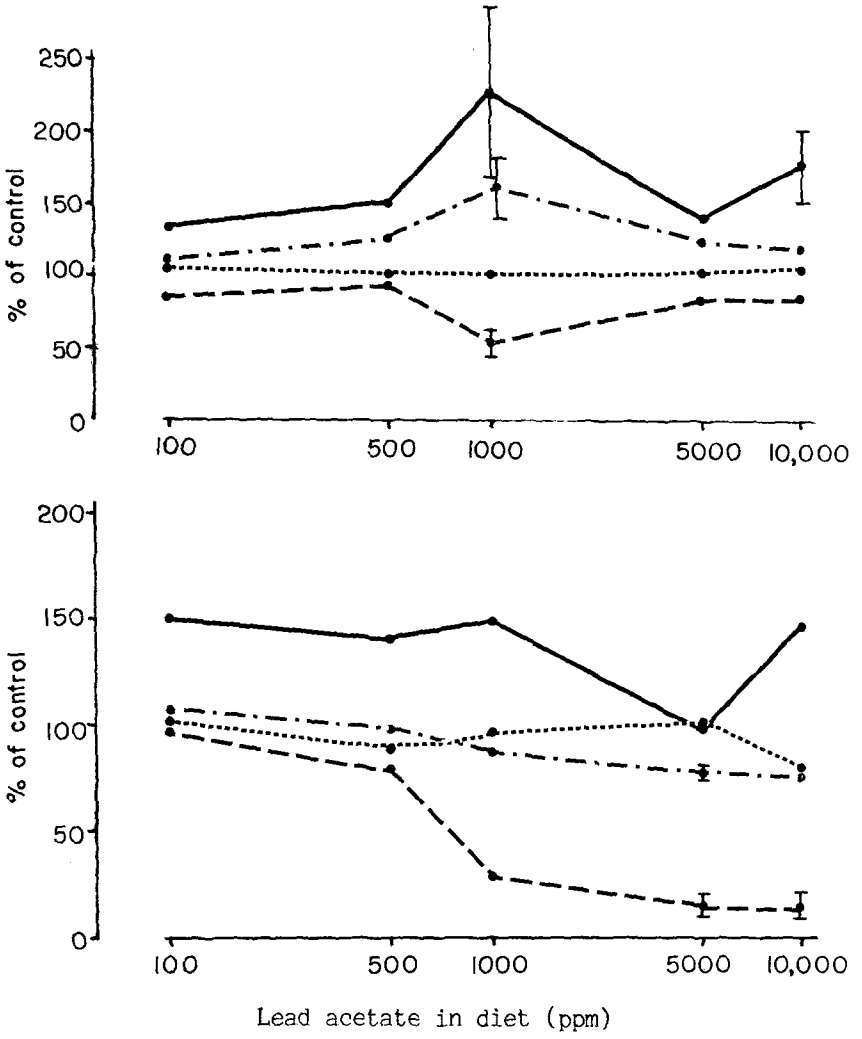

Figure 1. Liver microsomal enzyme activities, relative liver weight, body weight gain, and feed consumption for rats fed diets containing lead acetate for 15 days. Standard error of the mean illustrated by vertical bars only for those values significantly ($P<0.05$) different from control values.
Legend: Upper: —— EPN detoxication; .-.- O-demethylase; ..... relative liver weight; - - - hexobarbital sleep time.
Lower: —— cytochrome P-450; .-.- feed consumption; ..... microsomal protein; - - - body weight gain.

TABLE 1

Alterations in Hepatic Microsomal Enzyme Activity, Liver Weight, and Body Weight by Dietary Lead Acetate, Phenobarbital Sodium, and the Combination of These in Rats Fed Experimental Diets for 15 Days[a]

Added, ppm		Body Wt Gain	Feed Consumption	Liver Wt	Liver Microsomal Enzyme Activities		
Lead Acetate	Phenobarbital Sodium	(% of body wt)	(% of body wt)	(% of body wt)	Hexobarbital Sleep Time (min)	EPN Detoxication (μmoles PNP/ hr/g liver)	O-Demethylase (μmoles PNP/ hr/g liver)
0[b]	0[b]	45±6	147±10	5.1±0.1	107± 8	0.19±0.02	0.61±0.07
1000[c]	0[c]	25±4	121±16	5.3±0.1	53±14	0.24±0.08	1.06±0.07
0[b]	500[b]	56±4	168± 6	6.4±0.1	4± 1	0.72±0.07	1.71±0.34
1000[c]	500[c]	42±3	146± 4	7.0±0.2	1.0±0.2	1.07±0.07	3.15±0.13

Analysis of Variance

Source of Variation	Mean Squares					
Lead acetate[d]	1800**	2636**	0.600**	4250**	0.187**	3.958**
Phenobarbital[d]	1250**	2302**	10.200**	23300**	1.968**	10.665**
Lead acetate x phenobarbital[d]	305	12	0.120	910*	0.097*	1.076*
Within groups[e]	109	426	0.065	179	0.016	0.185

[a]Values are mean ± SE. [b]Five rats per group. [c]Four rats per group. One rat died during hexobarbital sleep time. [d]DF = 1. [e]DF = 14. *Significant at $P<0.05$. **Significant at $P<0.01$.

was also slightly elevated in livers of rats fed lead acetate at the 10,000 ppm level. The apparent elevations in concentrations of cytochrome P-450 and microsomal protein were not significant at the 0.05 level of probability because of the large variability of responses in each of the groups of rats fed lead acetate. Liver weight was unaffected by consumption of any level of lead acetate. In spite of depressed body growth in rats fed the higher levels of lead, there were no deaths in this experiment.

In the second experiment (Table 1), the effects of 1000 ppm lead acetate on liver microsomal enzyme activities were synergistic with those of 500 ppm phenobarbital sodium, i.e., the increases in enzyme activities for rats fed the combination of lead and phenobarbital were significantly greater than the sum of the increases due to feeding lead or phenobarbital singly. In other words, the stimulatory effects of phenobarbital sodium on detoxication activity were enhanced by adding lead acetate to the diet. Also, the ability of the microsomal system to be stimulated by lead was enhanced by phenobarbital. The depressions in weight gains and feed consumption associated with feeding lead acetate alone were virtually eliminated by adding phenobarbital to the diet. The effect of lead on liver weight in the second experiment would seem to be different from that seen in the first experiment. However, most of the effect on liver weight was due to the combination of lead and phenobarbital rather than lead alone.

DISCUSSION

There might seem to be apparent contradictions in the observations made by various investigators on the effects of lead compounds on hepatic detoxication activity. Some have reported that lead depressed this activity, while others observed no effect of lead on detoxication. In the present report, there are indications of enzyme enhancement. However, in most studies the changes in detoxication activity, whether inhibition or stimulation, were of low magnitude. Also, the capacity of microsomal enzymes to be stimulated in those studies by other enzyme inducers such as phenobarbital either was not impaired or was impaired only briefly. It is not possible from the research reported to fully define all conditions which determine the effect of lead on detoxication enzyme activity. Dosage of lead is certainly one factor which influences the response. Another factor is the time between exposure and sampling. In addition to dosage and time, other biologic and environmental variables such as age, strain, sex, species, and temperature must be considered in fully understanding biologic response to lead exposure.

The mechanism of stimulation of microsomal enzymes by lead is not fully known. One of the most appealing theories is that cytochrome P-450 levels rise because lead causes an accumulation of heme precursors which are utilized in enhanced cytochrome synthesis (EGAN & CORNISH 1973). Other ideas have been theorized by WEBB (1966) to explain examples of enzymic stimulation by heavy metals. It is even possible in feeding studies for part of the enzyme stimulatory action of lead to occur

in the diet. Heavy metal ions catalyze rancidity in feed, and rancid feed can enhance activity of microsomal enzymes (BROWN et al. 1954). Despite uncertainties about mechanisms, we conclude that, in rats fed a diet containing lead acetate the liver microsomal enzyme system continues to function and adapt to changes in exposure levels of foreign compounds.

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