# Enhancement of Hepatic Detoxification Enzyme Activity by Dietary Mercuric Acetate

by D. J. WAGSTAFF
*Department of Veterinary Physiology and Pharmacology*
*University of Missouri, Columbia, Mo. 65201*

Mercurials and other heavy metal compounds usually inhibit enzymic and metabolic processes (WEBB 1966). This inhibition is presumed to be the mechanism of intoxication by these compounds. Inhibition of detoxication activities of liver microsomal enzymes could be particularly determinetal because the toxicity of many nonmetal compounds would be increased. Such inhibition of microsomal enzymes has been observed after addition of mercurials to an *in vitro* assay system and in liver tissue taken from rats and mice pretreated with mercurials (CORNISH *et al.* 1970, RIBEIRO 1971). Contrary to this I have observed enhancement of microsomal enzyme activity in rats fed lead acetate for fifteen days. Because most compounds studied which stimulate microsomal enzymes are fat soluble (CONNEY 1967), stimulatory activity of water soluble heavy metal compounds is somewhat anomalous. The present report deals with stimulation of liver microsomal enzymes by dietary mercuric acetate (HgAc) and interactions of HgAc with phenobarbital sodium (PB).

## Methods

Experimental diet was fortified with 0, 100, 500, 1000, 2000, or 5000 ppm HgAc and was fed *ad libitum* to 100-110 g female Holtzman rats for fifteen days. Five rats in individual stainless steel wire mesh suspended cages were fed each dosage. The basal diet was vitamin A test diet purchased from Nutritional Biochemicals Corporation which was fortified with 0.69 ppm vitamin A acetate. In a second experiment, interactions of HgAc and PB were studied in a complete randomized design with factorially arranged treatments. Four groups of five rats each were fed diets fortified with a) no HgAc and no PB, b) 500 ppm HgAc and no PB, c) no HgAc and 500 ppm PB, and d) 500 ppm HgAc and 500 ppm PB.

Two toxicants, the insecticide EPN (O-ethyl-O-p-nitrophenyl phenylphosphonothioate) and p-nitroanisole were arbitarily selected for measuring detoxication activity. After fifteen days feeding each rat was killed with ether and the liver excised. A one g portion of liver was added to four ml of cold 1.15% KCl and ground in a glass-teflon homogenizer. The liver homogenate was centrifuged at five degrees C for fifteen minutes at 9000 x g. The supernatant fraction was assayed by methods of KINOSHITA *et al.* (1966) for oxidative cleavage of EPN (EPN detoxication) and O-demethylation



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of p-nitroanisole (O-demethylase). Product of both assays was p-nitrophenol (PNP).

## Results

There was increased detoxication activity in liver from HgAc-fed rats (Figure 1). In general, detoxication activity increased as the dietary dosage of HgAc was increased. Liver weight was unaffected by ingestion of HgAc. Toxicity of HgAc increased with dosage. The depressions in weight gain and feed consumption are illustrated in figure 1. There were no deaths among animals fed diets of 2000 ppm HgAc or less but all five animals fed the diet of 5000 ppm died after five but before ten days on the experiment.

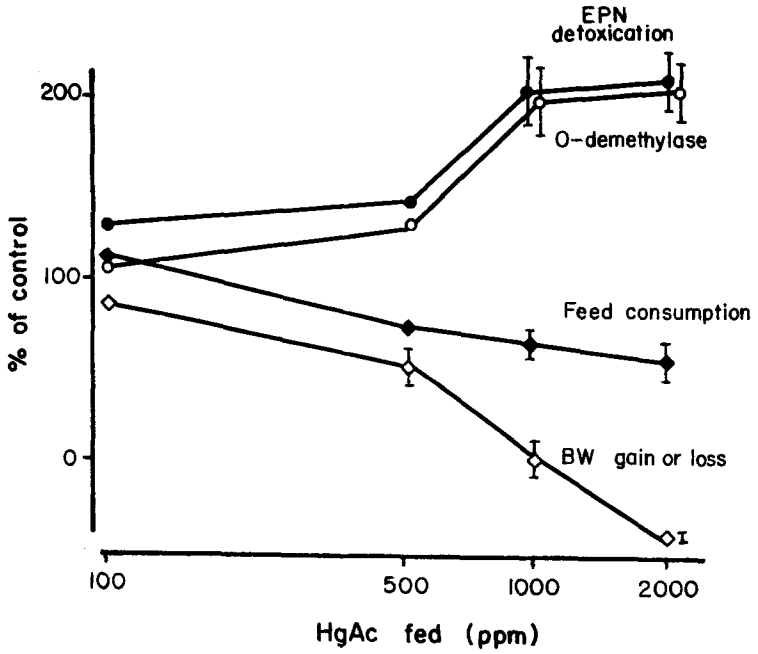


Figure 1

*Effects of dietary mercuric acetate (HgAc) on selected detoxication and intoxication responses in rats fed fifteen days on the experimental diet. Standard error of mean shown by vertical bars only for values significantly (P<.05) different from control values.*

In the second experiment, illustrated in Table 1, the stimulatory effects on microsomal activity of PB were synergized by HgAc. The HgAc administered singly at 500 ppm of the diet did not significantly increase activity of either detoxication procedure. The main effects of HgAc were statistically significant in the analyses of variance but it can be seen from table 1 that this was due almost wholly to the interaction with PB. The PB administered singly at 500 ppm of the diet had a marked effect on both detoxication procedures. When HgAc and PB were fed in the same diet the magnitude of the increases in detoxication activity were significantly greater than the sum of the increases for these compounds administered singly (note the PB X HgAc terms in the analyses of variance of Table 1). The depressions of feed consumption and body weight gain in HgAc-fed rats were similar to those illustrated in figure 1 and were not affected by concurrent feeding of PB. Feeding of PB singly increased liver weight and this effect was not altered by adding HgAc to the same diet.

Table 1

Effects of fifteen day feeding of mercuric acetate (HgAc) and phenobarbital Na (PB) on liver microsomal enzyme activity.

| treatment and dietary conc. (ppm) HgAc | PB | O-demethylase (μmoles PNP/ g liver/hr) | EPN detoxication (μmoles PNP/ g liver/hr) |
|---|---|---|---|
| 0 | 0 | .69 ± .04[a] | .20 ± .02 |
| 500 | 0 | .70 ± .07 | .34 ± .07 |
| 0 | 500 | 1.64 ± .30 | 1.07 ± .11 |
| 500 | 500 | 2.66 ± .34 | 1.74 ± .15 |

*Analyses of variance*

| SV | df | Mean squares | |
|---|---|---|---|
| HgAc | 1 | 1.3215* | .8181** |
| PB | 1 | 10.5314** | 6.3924** |
| PB X HgAc | 1 | 1.2545* | .3404* |
| Within groups | 16 | .2681 | .0472 |

[a]mean ± SE
*Significant at P<.05
**Significant at P<.01

## Discussion

There is a diphasic response of microsomal enzymes in animals exposed to mercurials. Rat exposed for three days had depressed activity (CORNISH *et al.* 1970) but in the present study there was enhancement after a fifteen-day exposure.

The stimulatory effect of HgAc on detoxication activities would seem to be indirect. The relationship of depressed body growth to increased detoxication activity illustrated in Figure 1 may suggest to some the enhancement of enzyme activity was due to a general response to stress. However, the synergism of the effects of PB and HgAc was not accompanied by any effect on growth rate in addition to the effect of HgAc administered singly. Webb (1966) postulated a number of mechanisms for enzyme stimulation by heavy metals, but further research is needed to determine their application to detoxication activities of liver microsomal enzymes.

The dosages of mercury used in these experiments were much above levels in the general environment. However, exposure to high concentrations do occur. In addition, the mercury-phenobarbital interactions support speculation that mercury in combination with other chemicals in the environment may have enzyme stimulatory capacity at low exposure levels.

Mercury tolerance or resistance result from genetic selection or adaptation of the individual. Strains of microorganisms have evolved which are highly resistant to mercurials (ROBERTSON 1943, IMSHENETSKY and PEROVA 1957, LOUTIT 1970, MAEDA *et al.* 1969, FURUKAWA *et al.* 1969, NAKAGAMI *et al.* 1969, SUZUKI *et al.* 1968, TONOMURA *et al.* 1968, TONOMURA 1968), but this process does not support a bright prognosis for higher species, especially mammals. Fortunately, the individual animal can adapt to increased mercurial exposure (GIL Y GIL 1924, TSURUMAKI *et al.* 1928, HUNTER 1929, MACNIDER 1941, SURTSHIN *et al.* 1958). Certain other compounds aid this adaptation. Rats pretreated with spironolactone, a nonhormonal catatoxic steroid (SELYE 1969, SELYE 1970a) possessing microsomal enzyme-stimulatory capacity (GERALD and FELLER 1970, BUCK and LAGE 1971, SOLYMOSS *et al.* 1970), were protected against doses of $HgCl_2$ which were lethal to nonpretreated animals (SELYE 1970b). The question arises as to the role of enhanced activity of liver microsomal enzymes in adaptation of animals to mercurials. Probably the observations presented here regarding a mercurial represent only one facet of the process whereby organisms adapt to an unfriendly chemical environment.

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