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Changes in Serum Enzymes after Inhalation Exposure of *p*-Xylene

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Serum enzyme activities are frequently used as indices of experimental hepatotoxicity. The use of serum enzyme activities in clinical test for hepatic damage has become a useful diagnostic and experimental tool (WILKINSON 1970, TIETZ 1970, GRICE et al. 1971). The changes in serum enzyme concentrations have been reported following exposure of animals to organic solvents (KORSRUD et al. 1972, CARLSON 1974). In recent years a number of investigators (WILKINSON 1970, GRICE et al. 1971) have studied the enzyme originating in the cell sap or mitochondria that appear in the serum during tissue damage. The transaminases appear to be the most useful enzyme for this purpose.

p-Xylene is used as a solvent in a number of industries. Recently it has been reported that there are some changes in blood chemistry of rats,cats and dogs following long-term exposure to vapors of mixed xylenes (CARPENTER et al. 1975). This study was initiated to evaluate the changes in serum enzyme activities which result from acute exposure of 1000, 1500 and 2000 ppm of p-xylene vapors. The enzymes studied normally have high activities in liver and low activities in the serum.

MATERIALS AND METHODS

Sprague-Dawley female rats (Charles River, Wilmington, Mass.) weighing between 200-250 g were used. Food and water were provided ad libitum except during the inhalation exposure.

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A single 4 hr inhalation exposure to p-xylene vapors was performed as described elsewhere (PATEL et al. 1978). Blood samples from two groups of 16 rats each were collected from the heart immediately after exposure and 24 hr after the beginning of the exposure. The blood samples were allowed to remain at 2-4°C for 1 hr until the serum could be removed.

Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) activities were measured as described by HENRY et al. (1960). The activities of glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione reductase (GSS-Rase) and isocitric dehydrogenase (ICD) were measured as described by BERGMEYER (1963). Enzyme assays were carried out at 340 nm with Gilford model 2400 recording spectrophotometer fitted with a constant temperature circulating bath at 37°C in a total volume of 2.0 ml.

5'-Nucleotidase activity was measured as described by DIXON and PURDON (1954) and estimation of phosphorous was done according to the method of FISKE and SUBBAROW (1925). Pseudocholinesterase activity (thereafter referred to as cholinesterase, 'AcChase') was measured as described by RAPPAPOTS et al. (1959).

RESULTS

The results of all the serum enzyme activities are shown in Fig. 1 and 2. The SGOT activity was not changed when measured immediately following termination of the 4 hr exposure to 1000, 1500 or 2000 ppm p-xylene. SGOT activity was significantly increased when measured 24 hr after the beginning of the exposure. The increase in enzyme activity was dose related. SGOT activity in control rats (exposed to air) was constant throughout the experiments. The SGPT activity was significantly increased immediately after 4 hr exposure to 1500 and 2000 ppm p-xylene. This activity was markedly increased after 24 hr at all three p-xylene concentrations.

Exposure to 1000 ppm p-xylene did not change the G-6-PDH activity in the 4 hr group but there was a slight increase in activity in the 24 hr group. G-6-PDH activity was markedly increased at both 4 and 24 hr after beginning of exposure to 1500 and 2000 ppm p-xylene. The increase of G-6-PDH activity was dose related.

The ICD activity was slightly below the control level immediately after the 4 hr exposure to 1000 ppm p-xylene and slightly above the control value 24 hr following the beginning of 4 hr exposure. When p-xylene

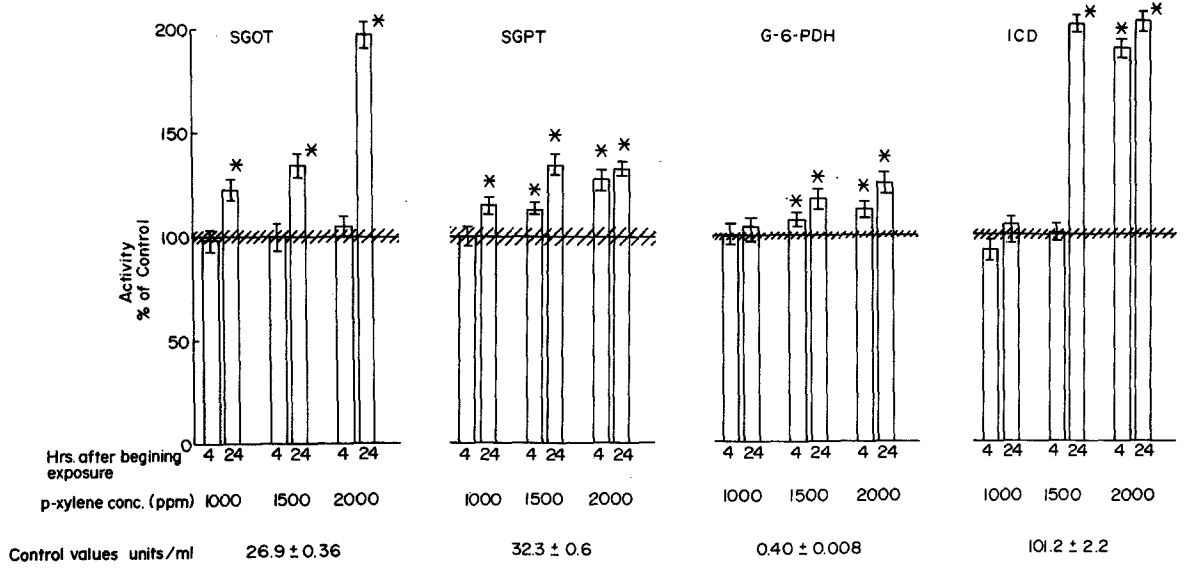

FIGURE 1. Serum enzyme activities in rats exposed to 1000, 1500 and 2000 ppm p-xylene.
*P<0.05

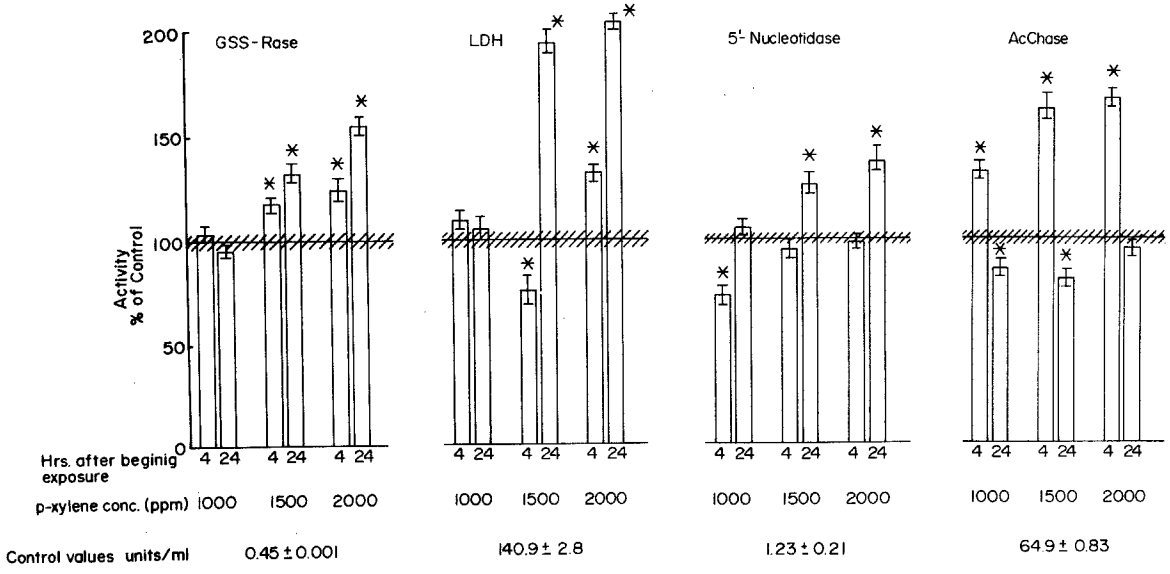

FIGURE 2. Serum enzyme activities in rats exposed to 1000, 1500 and 2000 ppm p-xylene.
*P<0.05

concentration was increased to 1500 ppm there was no change in ICD activity at 4 hr but a significant increase after 24 hr. ICD activity was markedly increased at both 4 and 24 hr after exposure to 2000 ppm p-xylene. A dose related response was indicated.

Exposure to 1000 ppm p-xylene did not change GSS-Rase activity. Dose and time related increases were observed at 4 and 24 hr of 1500 and 2000 ppm p-xylene exposure.

Exposure to 1000 ppm p-xylene caused a small increase of LDH activity at 4 and 24 hr. LDH activity was significantly lower immediately after the 4 hr exposure to 1500 ppm but markedly increased after 24 hr. This activity was elevated in both the 4 and 24 hr groups when animals were exposed to 2000 ppm p-xylene.

5'-Nucleotidase activity was significantly decreased immediately following the 4 hr exposure to 1000 ppm p-xylene; but, this activity was slightly above the control level after 24 hr. When the animals were exposed to 1500 and 2000 ppm the 5'-nucleotidase activity was unchanged in the 4 hr group but significantly elevated in the 24 hr group. A dose related response to p-xylene exposure was observed in rats only when there was a 24 hr lapse between the beginning of the 4 hr exposure on the assay for 5'-nucleotidase activity.

There was a dose related elevation of AcChase activity 4 hr after the p-xylene exposure at all three concentrations. These activities reverted to levels below the control value at 24 hr.

DISCUSSION

When rats were exposed to the different concentrations of p-xylene vapors, some serum enzyme activities were modified in a dose related fashion. Significant increase in the NADPH formation through the intermediate metabolic pathways suggest that there is an alteration of liver fuction as well.

Toxicity of isomers of xylene in man has been known for 40 years (DE OLIVERIA 1936). WORONOW (1929) found rise in white cell count in rabbits after subcutaneous injection of xylene in oil. A similar observation has recently been reported in rats after inhalation (CARPENTER et al. 1975). Many investigators belived at that time that metabolism of this solvent may reduce the toxicity. Several researchers investigated metabolism and reported that all isomers of xylene are transformed by oxidation of one of the methyl group to the corresponding toluic acid (FABRE et al. $1960_{a,b}$).

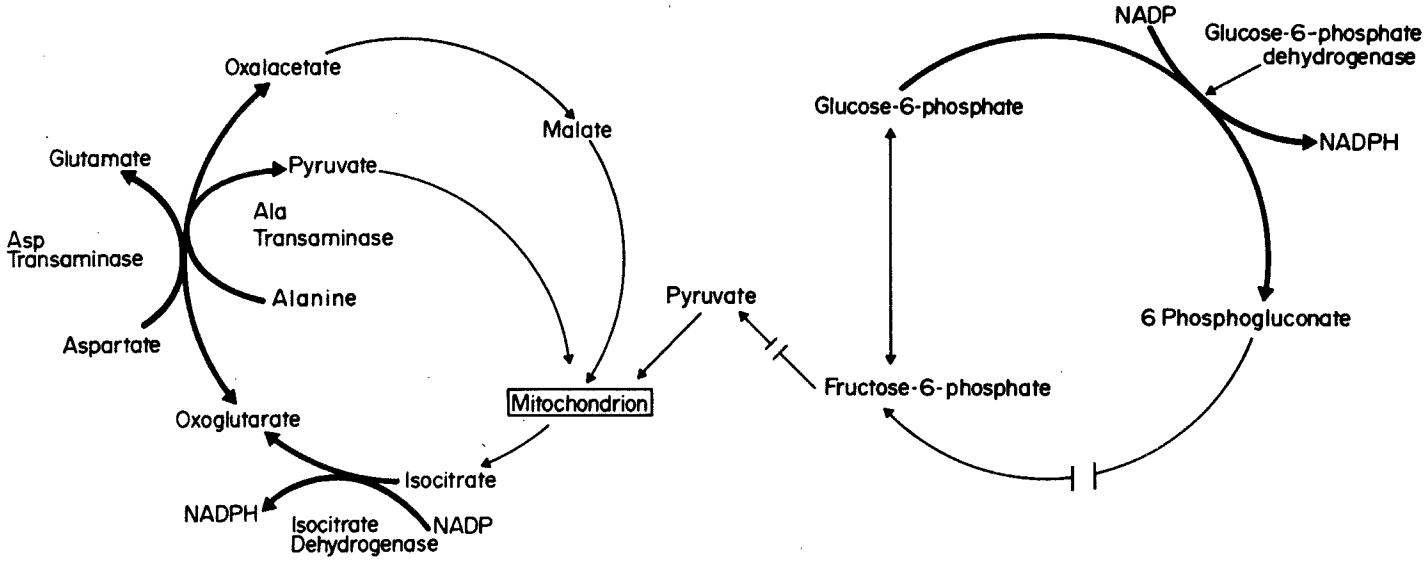

FIGURE 3. Effect of p-xylene exposure on NADPH production through increased intermediary metabolism of glucose and amino acids.

Most of these studies have been done with mixed xylenes having 8% p-xylene. According to LAZAREW (1929) p-xylene is the most toxic isomer of the mixed xylene. None of these studies explain the complete metabolism and biotransformation of this solvent nor the mechanism of toxicity. Recently CARLONE and FOUTS (1974) reported that p-xylene is metabolized in vitro by rabbit lung and liver microsomes in the presence of hepatic soluble enzymes. It has also been reported that the hepatic tissue is involved in detoxication of this solvent (PATEL et al. 1978).

In the present study we looked at several serum enzymes which are believed to originate in the liver. The increase in SGOT and SGPT activities are indicative of hepatocellular damage. The increased activities of G-6-PDH and ICD suggest an increase production of NADPH with enhancement of intermediary metabolism with increased utilization of glucose and amino acids (Fig. 3). The increased NADPH production from elevated G-6-PDH may result in increased fatty acid synthesis. The dose dependent increase in GSS-Rase activity possibly effects the pathway by which tissue levels of GSH are maintained by providing the essential reduced cofactor as suggested in ozone toxicity by BUCKLEY et al. (1975).

The increased serum LDH activity in rats exposed to higher concentrations of p-xylene was possibly liberated from these damaged tissues. Dose dependent increase of the 5'-nucleotidase activity may be due to dysfuction in the hepato biliary tract. The time and dose dependent variation and sensitivity of AcChase activity may be due to the changes in RBC metabolism and/or adaptation.

We have previously reported that a metabolite produced in p-xylene metabolism had significant biochemical damage in the pulmonary tissue as early as 2 hr after administration of p-xylene (PATEL et al. 1978). Therefore, it is highly possible that changes in serum enzyme activities are caused by metabolites of p-xylene as well as the parent compound.

In many industries and laboratories, workers are exposed to p-xylene for longer periods of time although of very low concentrations. Further work is needed in the nature of subtle danage by chronic exposure to low concentrations of p-xylene.

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