Effects of Petroleum Hydrocarbon Solvents on Alkaline Phosphatase of Rats

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Environmental and occupational hazards due to petroleum hydrocarbon solvents are known. The detection of intoxication caused by derivatives of mineral oil is of paramount practical significance for workers employed in refineries, petrochemical industries, tanker and transport fleets. Some of the petroleum derivatives cause liver injury, and disturbance in haematopoietic and central nervous systems (BROWNING, 1965, AYELSON et al., 1976).

The possible mode of toxic action of industrial solvents remains still obscure in spite of extensive investigations (WEAVER, 1972). As part of a programme for identifying biochemical parameters for the detection of incipient toxicity of petroleum solvents, the alteration in alkaline phosphatase activities in serum, liver and kidney was investigated in rats.

Materials and Methods

Male adult albino rats (150-200 g) drawn from I.T.R.C. stock colony, maintained on standard Hind Lever pellet diet were divided into 14 groups, 10 rats per group. Benzene (A.R., B.D.H.) CCl_4 (A.R., B.D.H.), gasoline, petroleum ether (A.R., B.D.H., B.P. 40-60°C) or IOMEX were administered intraperitoneally daily in doses of 3 ml of each solvent/kg body weight in each group except for the first two groups which received 2 ml/kg. IOMEX is a wide spectrum weedicide, a petroleum product rich in aromatics, marketed by Indian Oil (RAO et al., in press). The control group received only normal saline. At the end of the prescribed period animals were sacrificed and homogenates were prepared in 0.25 M sucrose using a Teflon homogenizer.

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Alkaline phosphatase (E.C. 3.1.3.1) - The activities of alkaline phosphatase in various tissues homogenate and serum were determined using disodium phenyl phosphate as the substrate. The liberated phenol were measured according to the method of WOOTTON (1964).

(A) Method for differential separation of alkaline phosphatase activity - The differential separation of these activities by addition 50% ice cold ethanol to serum of control and experimental animals according to the following procedure: 0.5 ml of 50% ice cold ethanol was added slowly to 0.5 ml of serum in cold. After 10 minutes precipitates from the sera were removed by centrifugation at 2,000 rpm for 10 min. in the cold and an aliquot quantity of supernatant was assayed for enzymic activities.

Protein - Protein was estimated according to method of LOWRY et al. (1951).

Polyacrylamide gel electrophoresis - Polyacrylamide gel electrophoresis was performed according to the procedure of HODSON et al. (1962). After application of aliquot quantities (50/µl) of samples, electrophoresis was performed at 0-4°C at a constant voltage 200 V for a period of 2 hrs. Visualization of alkaline phosphatase activity was achieved by coupling of liberated napthol from napthyl phosphate, with diazonium salt fast red T.R.

Results

The results of alkaline phosphatase activities of various organs after the administration of petroleum ether, benzene, gasoline and IOMEX are given in Table 1. The alkaline phosphatase activity of the liver was significantly increased ($P<0.01$) in all the animals of four hydrocarbon solvent administered groups. The percentage increases were 182, 166, 228 and 122 in 2 days and 216, 202, 411 and 278 at 7 days after benzene, gasoline, petroleum ether and IOMEX, respectively. There was no significant change in brain alkaline phosphate activity for any of these groups. Serum alkaline phosphatase showed a slight decrease. On the other hand, the kidney alkaline phosphatase activity of these groups showed a significant decrease ($P<0.01$). The decreases were to the extent of 60, 54, 50, 60% in 2 days and at 7 days 50, 46, 50, and 60% after benzene, gasoline, petroleum ether and IOMEX administration, respectively.

Table 1

Effect of administration of benzene, petroleum ether, gasoline, IOMEX on alkaline phosphatase (n moles of phenol liberated/min/mg protein or/ml serum) of liver, brain, kidney and serum of rats after 2 and 7 days.

Group	Benzene				Petroleum ether			
	Liver	Brain	Kidney	Serum	Liver	Brain	Kidney	Serum
Control	5.6±0.05	12.1±0.3	240.1±0.3	3.9±0.06	5.7±0.05	10.2±0.3	209.8±1.8	4.1±0.08
2 days	10.2±0.17[a]	13.1±2.0	144.2±2.9[b]	3.0±0.70	13.0±0.14[a]	11.3±0.21	104.9±2.2[a]	2.3±0.06[a]
7 days	12.1±0.17[a]	12.6±1.1	119.5±2.7[a]	3.3±0.06[b]	23.4±0.28[a]	12.0±0.24[c]	104.9±2.3[a]	2.0±0.05[a]

Group	Gasoline				IOMEX			
	Liver	Brain	Kidney	Serum	Liver	Brain	Kidney	Serum
Control	5.6±0.05	11.6±0.28	240.1±1.8	3.9±0.06	5.1±0.1	10.1±1.1	248.4±1.5	4.0±0.5
2 days	9.3±0.09[a]	11.3±0.31	129.2±2.0[a]	3.0±0.08[b]	6.2±0.1[b]	10.6±1.2	149.0±2.0[a]	3.5±1.0
7 days	11.3±0.13[a]	10.4±0.32[c]	110.9±1.0[a]	3.2±0.07[b]	14.2±0.6[a]	11.1±2.3	164.2±1.9[a]	3.5±0.5

Values represent the mean ± S.E. of ten estimations.

a = $p < .001$, b = $p < .01$, o = $p < .05$.

The isoenzyme pattern of liver, kidney and serum of control and experimental animals is shown in Fig. 1. There

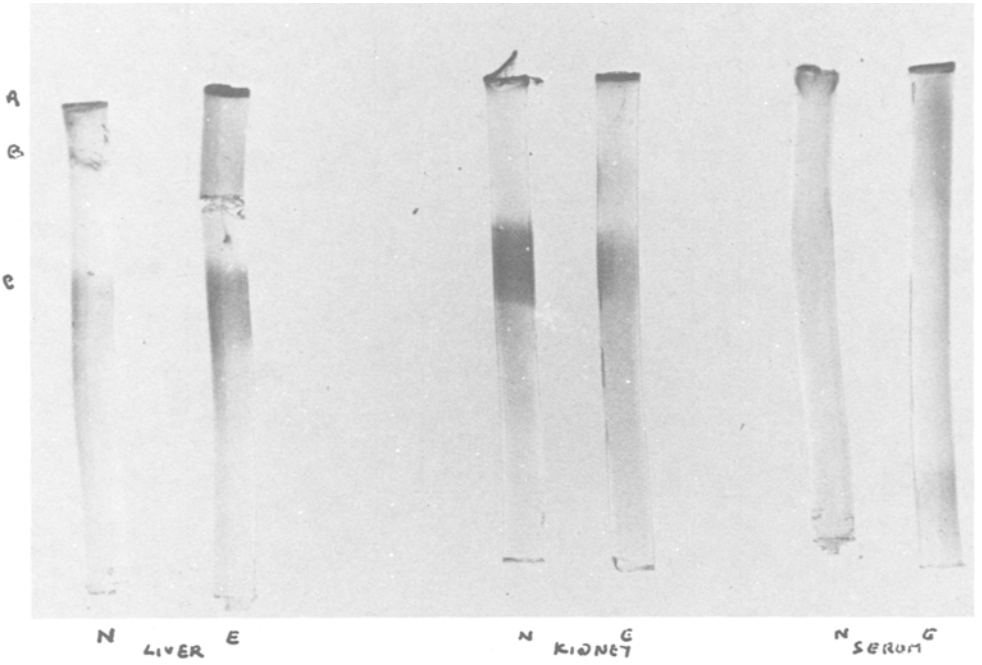

Figure 1. Polyacrylamide gel electrophoresis showing alkaline phosphatase isoenzymes of liver, kidney and serum of control (N) and experimental rats at the end of 7 days.

was a slight difference in mobility of main isoenzyme bands of liver and kidney. The main isoenzyme band of kidney moved more slowly. A remarkable increase in liver isoenzyme bands was observed in the experimental group compared to that of control group of animals. On the other hand, the main isoenzyme band of kidney showed a decrease in the intensity. As the amount of activity in the serum bands was less, it was difficult to assign an origin to a particular band.

Table 2

Effect of addition of 50% alcohol on serum alkaline phosphatase activity (n moles of phenol liberated/ml) of control and experimental groups (at the end of 7 days) of rats.

Groups	Activity in serum before alcohol addition ± S.E.	Activity in supernatant after addition of alcohol ± S.E.	Percent change
Control	4.14±0.17	3.55±0.21	85.57
Benzene	3.30±0.06	4.50±0.05	136.36
Gasoline	3.20±0.17	4.80±1.10	150.00
IOMEX	3.50±0.12	4.65±0.14	132.86
CCl_4	5.40±0.20	5.00±0.20	92.59

Table 2 gives activities of serum and 50% ethanolic supernatant of the serum in control and experimental groups. In control group of animals 86% of the original activity remained in supernatant. While in benzene, gasoline and IOMEX (hydrocarbon solvents) showed a significant increase in activity (132-150%) but the chlorinated hydrocarbon group (CCl_4) did not show corresponding increase in the activity.

Discussion

Hepatic microsomal mixed function oxidase involving molecular oxygen, NADPH and cytochrome P450 is primarily responsible for the metabolism of benzene and many other xenobiotics. The mechanism of hydroxylation by the mixed function oxidase has not yet been completely determined. JERINA and DALY (1974) suggested that the reaction probably occurred via the formation of an epoxide intermediate. However, the role of alkaline phosphatase in the metabolism and toxicity of hydrocarbon solvents is not yet clear. AMBROSIO (1942) reported alteration in phosphatase activity in benzene poisoning. BIONDI (1956) observed progressive reduction in alkaline phosphatase in W.B.C. in sub-acute poisoning. ROZERA (1960) has observed reduction in blood

serum alkaline phosphatase and acid phosphatase after intramuscular administration of benzene. CARPENTER et al. (1975) noted slight initial decrease in activity after inhalation of some commerical hydrocarbon solvents. The present study also showed a slight decrease in the serum alkaline phosphatase activity after the administration of benzene, petroleum ether, gasoline and IOMEX. However, liver alkaline phosphatase activities showed a significant increase while kidney alkaline phosphatase showed a significant reduction indicating involvement of both organs. Previous studies of GRANATI (1958) and ROZERA (1960) did not show any histological changes but obtained little evidence of disturbed liver function after benzene administration. LEE et al. (1973) observed 50% inhibition of Fe^{59} incorporation into erythrocytes after administration of 2,200 mg/kg of the benzene indicating disturbance of the bone marrow.

The changes in alkaline phosphatase activities appear to be more sensitive in benzene and other hydrocarbon solvent-induced toxicity compared to depression in Fe^{59}- incorporation after the method of LEE et al. (1973). Even a single 1/10th of the present dose of the solvents showed similar changes in the liver alkaline phosphatase activities (GOEL et al., unpublished results).

This differential behaviour of the activity in various organs may be due to a variable effect of the toxicant on the different isoenzymes or may be due to presence of different metabolites. It appears from gel electrophoresis that kidney and liver do have different isoenzymes, with different mobilities. In the experimental group there is increase in one of liver isoenzyme bands while in the kidney there was decrease in one of the bands.

Increase in activities of serum supernatant alkaline phosphatase after addition of 50% alcohol in the hydrocarbon solvent groups may be due to (a) presence of liver isoenzyme in ethanolic supernatant, or (b) removal of some inhibitor by alcohol.

PEACOCK et al. (1963) observed that in the presence of 20% ethanol most of the phosphatase activity in the sera of patients with bone disease was precipitated, whereas in liver disease the enzyme activity remained in the supernatant. It is possible that kidney isoenzyme in serum which is already decreased in activity was precipitated by 50% ethanol.

Supernatant may contain mainly liver isoenzyme which is already increased under experimental conditions. It is also possible that ethanol which is more polar than hydrocarbon solvents displaces the non-polar inhibitor from the receptive site and hence the increase in activity. Further work is necessary to clarify these points. The detection of this extra amount of activity in ethanol supernatant may provide a simple test for monitoring the toxicity induced by gasoline, petroleum hydrocarbon solvents and benzene. As there is no increase in activity of CCl_4 group, this test may exclude chlorinated hydrocarbon compounds.

Summary

After administration of benzene, gasoline, IOMEX or petroleum ether, liver alkaline phosphatase showed over 200% increase in activity whereas kidney alkaline phosphatase activity was depressed by 50%. The pattern of separation of isoenzymes of alkaline phosphatase in liver, kidney and serum indicated involvement of one of the isoenzymes. A simple method of detecting the incipent toxicity is described.

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