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EFFECT OF *n*-OCTANE AND *n*-NONANE ADMINISTRATION ON ALKALINE PHOSPHATASE ACTIVITY IN TISSUES OF FEMALE RATS

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Abstract—Studies on alkaline phosphatase were conducted in albino rats exposed to *n*-octane or *n*-nonane for 2 and 7 days; there was an increase in alkaline phosphatase activity of liver, spleen, and bone marrow. Increase in spleen alkaline phosphatase activity persisted up to 42 days after single dose of *n*-octane or *n*-nonane. Pretreatment with protein synthesis inhibitors, cycloheximide or ethionine removed this observed increase of alkaline phosphatase activity in liver and spleen.

Serum alkaline phosphatase is a well accepted diagnostic tool for many diseases, viz. hepatic disorders [1], and bone diseases [2, 3]. Intake of many drugs, chemicals and solvents results in the increase of hepatic alkaline phosphatase activity [4–6]. This increase in liver alkaline phosphatase is often associated with induction. Earlier it has been shown from our laboratory that treatment of rats with petroleum hydrocarbons leads to an increase of hepatic alkaline phosphatase activity [7–9]. In addition a single dose of benzene has been reported to cause an increase in hepatic alkaline phosphatase activity even within three hours of its administration [10]. The present studies were aimed to delineate the profile of alkaline phosphatase in various tissues after *n*-octane and *n*-nonane administration to female rats. Attempts have been made to elucidate the mechanism of elevation of liver and spleen alkaline phosphatase activity.

MATERIALS AND METHODS

Animals. Female adult albino rats (150 ± 10 g) drawn from I.T.R.C. Stock Colony, maintained on standard Hind liver pellet diet and water *ad lib.*, were used throughout the experiment.

Effect on various tissues. Twenty four animals were taken and divided into three groups, eight in each. Two groups received *n*-octane or *n*-nonane (Thomas and Baker, India; 99% chromatographically pure) intraperitoneally daily (1.0 ml/kg body wt) for 2 or 7 days and the third group which served as control received only normal saline. At the end of the treatment schedule, animals were killed and the tissues were placed over crushed ice; homogenates of liver, kidney, spleen and brain (10% w/v) were prepared in cold 0.25 M sucrose using a Potter–Elvejhem type glass homogenizer fitted with Teflon pestle [22].

For studying effect of single dose, 90 animals were taken and divided into 3 groups. Two groups received a single dose of *n*-octane or *n*-nonane (1.0 ml/kg body wt) and the third control group received a single dose of normal saline. At different days as indicated 5 animals were sacrificed from each group.

Studies with cycloheximide and ethionine. Fifty-six animals were taken and divided into 7 groups of 8 rats each. Cycloheximide or ethionine (both from Sigma Chemical Co., St. Louis, MO) were given intraperitoneally at the dose of 1 mg or 70 mg/kg body wt, respectively, 20 min prior to *n*-octane or *n*-nonane administration (1.0 ml/kg body wt, intraperitoneally).

Enzyme assay. Alkaline phosphatase (EC 3.1.3.1) activity in various tissue homogenates and serum were assayed using disodium phenyl phosphate as the substrate and the liberated phenol was measured according to the procedure of Wooton [11].

Protein. Protein was estimated according to the method of Lowry *et al.* [12], using bovine serum albumin as standard.

RESULTS

The levels of alkaline phosphatase activity of various organs after the intraperitoneal administration of *n*-octane or *n*-nonane for 2 or 7 days are given in Table 1. The alkaline phosphatase activities of liver, spleen and bone marrow were significantly increased after 2 and 7 days of exposure. However, change in brain alkaline phosphatase was not appreciable. Serum alkaline phosphatase activity was significantly decreased after 2 days of exposure with a much more marked increase after 7 days of treatment. On the other hand, kidney alkaline phosphatase activity showed a significant decrease both 2 and 7 days after administration of the solvents (Table 1).

Enhanced activity in alkaline phosphatase of spleen persisted even after 42 days after single intraperitoneal administration of solvents (Table 2).

Studies with cycloheximide and ethionine. Results given in Table 3 indicate that the increase in the activity of alkaline phosphatase as a result of exposure to *n*-octane or *n*-nonane is blocked by the two protein synthesis inhibitors cycloheximide and ethionine.

DISCUSSION

Administration of petroleum hydrocarbon solvents like benzene, gasoline or iomex has been reported to lead to an increase of hepatic alkaline phosphatase activity [8]. In this study it is observed

Table 1. Effect of administration of *n*-octane or *n*-nonane on alkaline phosphatase (nmoles phenol liberated/min/mg protein or ml serum) daily for 2 or 7 days in albino rats

	Liver	Kidney	Spleen	Brain	Bone marrow	Serum
After 2 days						
Control	2.75 ± 0.20	568.24 ± 31.42	18.23 ± 3.06	9.23 ± 1.23	186.27 ± 8.68	126.00 ± 11.60
n-Octane	8.58 ± 0.56*	302.29 ± 28.87‡	39.29 ± 2.36*	9.55 ± 1.03	258.36 ± 10.49†	54.04 ± 11.09*
n-Nonane	14.06 ± 1.16*	305.26 ± 23.45†	42.93 ± 3.06*	9.98 ± 0.95	266.43 ± 13.23†	78.00 ± 11.60*
After 7 days						
Control	2.76 ± 0.03	543.84 ± 35.26	18.46 ± 3.07	9.22 ± 0.92	180.86 ± 9.34	116.00 ± 11.60
n-Octane	24.26 ± 3.69*	208.15 ± 21.27*	56.01 ± 2.36*	10.06 ± 0.82	552.75 ± 29.88*	215.03 ± 11.10*
n-Nonane	23.48 ± 2.40*	217.30 ± 19.18*	58.30 ± 2.13	9.92 ± 0.96	572.04 ± 20.60*	247.00 ± 12.03*

Values expressed in units ± S.E.
P values: * = < 0.001, † = < 0.01, ‡ = < 0.02.

Table 2. Effect of single intraperitoneal administration of *n*-octane or *n*-nonane (1.0 ml/kg body wt) on alkaline phosphatase (nmoles phenol liberated/min/mg/of protein of liver, kidney and spleen of albino rats

	1 Day	10 Days	20 Days	26 Days	42 Days
Liver					
Control	2.70 ± 0.21	2.71 ± 0.49	2.76 ± 0.21	2.76 ± 0.50	2.66 ± 0.22
n-Octane	4.58 ± 0.35†	2.79 ± 0.26	2.92 ± 0.58	2.62 ± 0.46	2.76 ± 0.27
n-Nonane	6.47 ± 0.66*	2.77 ± 0.26	2.92 ± 0.41	2.94 ± 0.58	2.65 ± 0.28
Kidney					
Control	547.34 ± 21.01	604.10 ± 32.12	558.30 ± 25.58	604.46 ± 26.75	593.97 ± 26.20
n-Octane	427.62 ± 28.21‡	601.49 ± 22.49	505.15 ± 27.65	605.65 ± 26.88	558.42 ± 19.47
n-Nonane	343.50 ± 27.23†	613.46 ± 21.67	528.82 ± 29.63	605.14 ± 22.73	569.14 ± 20.26
Spleen					
Control	18.10 ± 1.30	17.30 ± 1.67	17.30 ± 2.03	17.58 ± 2.68	18.29 ± 1.19
n-Octane	57.83 ± 1.75*	53.86 ± 6.43*	48.24 ± 6.43*	46.78 ± 2.28*	43.68 ± 2.63†
n-Nonane	64.95 ± 1.72*	54.99 ± 6.74*	49.47 ± 1.07*	46.44 ± 3.27*	44.05 ± 3.00†

Values expressed in units ± S.E.
P values: * = < 0.001, † = < 0.01 and ‡ = < 0.05.

Table 3. Effect of *n*-octane or *n*-nonane (1.0 ml/kg body wt) treatment (single dose) on alkaline phosphatase (nmoles phenol liberated/min/mg of protein) of liver and spleen pretreated with cycloheximide (1.0 mg/kg body wt) or ethionine (70 mg/kg body wt) of female albino rats

	Liver	Spleen
Control	2.70 ± 0.21	16.01 ± 1.30
n-Octane	5.78 ± 0.22*	35.66 ± 1.20*
n-Nonane	6.47 ± 0.66*	38.07 ± 2.54*
Cycloheximide	2.83 ± 0.30	18.90 ± 1.12
Cycloheximide + *n*-octane	2.89 ± 0.31	16.47 ± 0.96
Cycloheximide + *n*-nonane	3.59 ± 0.43	18.27 ± 1.31
Ethionine	2.60 ± 0.25	14.59 ± 0.46
Ethionine + *n*-octane	2.51 ± 0.16	14.91 ± 0.60
Ethionine + *n*-nonane	2.61 ± 0.07	12.86 ± 1.47

Values expressed in units ± S.E.
P values: * = < 0.001.

that the activity of alkaline phosphatase was also increased significantly in liver, spleen and bone marrow and decreased in kidney after administration of the aliphatic solvents *n*-octane and *n*-nonane for 2 and 7 days. 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 the presence of different metabolities in different organs.

The manner in which the hepatic alkaline phosphatase activity increased is not well understood. It may be due to *de novo* synthesis of enzyme or decrease in turnover rate of the enzyme, or due to other extraneous factors. There have been many examples of induction of alkaline phosphatase *in vivo*, some of which require *de novo* protein synthesis (induction) and some of which do not (enhancement). It has been found in man that the leukocyte alkaline phosphatase level is elevated during stress imposed by a diseased state [13]. Vitamin D has been found to increase alkaline phosphatase activity [14] in the brush border of the chick intestine, an event that is attributed to increased enzyme synthesis, since cycloheximide or actinomycin D eliminated this effect [15]. Liver alkaline phosphatase is induced by various chemicals [16, 19], by hormones [20], or in many liver diseases.

Thoenen [21] observed that increase in tyrosine hydroxylase could be prevented by cycloheximide and concluded that induction of enzyme synthesis was involved. Similarly, the present study indicates that the elevation in liver and spleen alkaline phosphatase activity after exposure to solvents is likely to involve induction which is blocked by protein synthesis inhibitors, viz. cycloheximide and ethionine. However, the effect of cycloheximide and ethionine on the enzyme is not clearly understood and requires further study.

Similarly alkaline phosphatase activity in liver, kidney, spleen, bone marrow and serum after ninety days intraperitoneal or dermal administration to rats with these solvents, was altered.

Earlier studies by Rao and Pandya [10] with benzene indicated that liver alkaline phosphatase activity and lipid peroxidation remains elevated for more than a week is also confirmed by present results. Increased activity of spleen alkaline phosphatase after single intraperitoneal injection of solvents remained up to 42 days, whereas hepatic alkaline phosphatase showed normal level within 10 days, may indicate that induced enzyme of spleen is much more stable than that of liver.

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