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Changes in Hepatic Carbohydrate Metabolism of the Mouse, *Mus booduga* (Gray), by Hexachlorophene Treatment

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Hexachlorophene (HCP) has been used extensively in consumer products, medical and agriculture preparations as an antibacterial and antifungal agent (Marzulli and Maibach 1975). But the safety of HCP use in some of these preparations has been questioned due to its potential neurotoxic effects (Powell and Lampert 1977). Some reports indicate binding of HCP to liver proteins and accumulation of high concentrations of HCP in liver (Powell and Lampert 1977). *In vitro* and *in vivo* inhibition of the enzymes of energy metabolism (Wang and Buhler 1978) and manifestation of hepatic mitochondrial lesions (Caldwell *et al.* 1972) by HCP, prompted us to study some metabolic consequences in *Mus booduga* liver during repeated HCP treatment.

MATERIALS AND METHODS

Six groups of healthy mice *Mus booduga* in the weight range of 10± 2 g (Mean, ± S.D.), fed *ad lib*, were aclimatized to laboratory conditions (temperature 30 ± 2°C; relative humidity 75 %; and a light period of 12 h) for ten days prior to experimentation. This species has been successfully used earlier in our laboratory as an animal model in toxicological studies (Prasad 1986).

Technical grade hexachlorophene (2, 2–methylenebis–3, 4, 6 –trichlorophenol) obtained from Sigma Chemical Co., USA, was dissolved in a minimum volume of corn oil and was administered orally at a dose of 60 mg/kg/day to the first three groups of mice for 1, 3 and 7 days respectively. The remaining three groups were given an isovolumetric amount (0.02 ml/day) of corn oil and were treated as controls. Mice were sacrificed on the 2nd, 4th and 8th day, and blood samples were obtained in heparinized vials by tail and cardiac punctures. Glucose (Kemp and Van Heijningen 1954), lactate (Barker and Summerson 1941) and pyruvate (Reitman and Frankel 1957) contents were analysed in the blood samples. The liver was excised rapidly and 10% (W/V) homogenate was prepared in 0.25 M ice cold sucrose using a Yorko–speed control homogenizer. The homogenate was centrifuged at 4000 X g for 20 min, and the cell free extract was used for the estimation of glygogen (Kemp and Van Heijningen 1954), lactate, pyruvate contents and for assaying lactate (LDH), succinate (SDH) and malate (MDH) dehydrogenases (Nachlas *et al.* 1960). The protein concentration in the enzyme source was analysed following the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

The enzyme activities and the concentration of the metabolites were expressed as the mean, ± S.D. of six observations. The significance of the difference between control and experimental samples was assessed by the student's t–test.

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Table 1. Changes in *Mus booduga* blood parameters during repeated hexachlorophene treatment

Parameter	Control	1 day	Control	3 days	Control	7 days
Glucose[1]	60.32 ± 8.65	64.92 ± 6.74 (+7.63)	65.91 ± 5.01	78.12 ± 5.11** (+18.53)	62.01 ± 2.98	82.46 ± 7.53** (+32.98)
Lactic acid[1]	9.53 ± 1.26	9.94 ± 0.96 (+4.30)	10.63 ± 0.96	17.57 ± 1.53** (+65.29)	10.23 ± 1.53	19.76 ± 2.04** (+93.16)
Pyruvic acid[1]	0.64 ± 0.04	0.58 ± 0.03* (-9.38)	0.73 ± 0.09	0.44 ± 0.05** (-39.73)	0.70 ± 0.05	0.43 ± 0.098** (-38.57)
Lactic acid/ Pyruvic acid	16.93 ± 1.98	19.36 ± 1.25* (+14.35)	18.64 ± 2.03	42.60 ± 4.32** (+128.5)	17.60 ± 2.96	48.16 ± 4.39** (+173.6)

[1] Values are represented as mg/100 ml of blood.

Values are means, ±S.D. of six experiments.

Values in the parentheses are % changes over controls.

* Significantly different from control $p<0.01$.

** Significantly different from control $p<0.001$.

Table 2. Changes in the levels of glycogen, lactic acid, pyruvic acid and lactic acid/pyruvic acid ratios of liver of mouse *Mus booduga* during repeated hexachlorophene treatment

Parameter	Control	1 day	Control	3 days	Control	7 days
Glycogen[1]	36.90 ± 4.34	34.78 ± 2.93 (-5.75)	.32.13 ± 4.93	22.41 ± 5.05* (-30.25)	34.56 ± 5.32	11.59 ± 3.19** (-66.46)
Lactic acid[1]	4.46 ± 0.69	4.99 ± 0.52 (+11.88)	4.92 ± 0.53	7.08 ± 0.46** (+43.90)	5.02 ± 0.73	7.63 ± 1.02** (+51.99)
Pyruvic acid[1]	0.82 ± 0.05	0.75 ± 0.04 (-8.54)	0.86 ± 0.03	0.81 ± 0.07 (-5.8)	0.91 ± 0.05	0.52 ± 0.12** (-42.86)
Lactic aicd/ Puruvic acid	4.96 ± 0.99	7.32 ± 1.03** (+47.58)	5.35 ± 0.54	9.74 ± 0.95** (+82.06)	4.72 ± 0.25	10.67 ± 1.47** (+126.1)

[1] Values are represented as mg/gm tissue.

Values are means, ± S.D. of six observations.

Values in parentheses are % changes over control.

* Significantly different fron control $p<0.01$.

** Significantly different from control $p<0.001$.

Table 3. Changes in the activity levels of hepatic lactate, succinate and malate dehydrogenases in *Mus booduga* during repeated hexachlorophene treatment.

Enzyme	Control	1 day	Control	3 days	Control	7 days
Lactate dehydrogenase	2.33 ± 0.08	2.41 ± 0.10 (+3.43)	2.25 ± 0.09	1.37 ± 0.04** (-39.11)	2.25 ± 0.06	1.45 ± 0.05** (-35.56)
Succinate dehydrogenase	0.578 ± 0.09	0.436 ± 0.08* (-24.57)	0.595 ± 0.06	0.501 ± 0.03* (-15.8)	0.583 ± 0.02	0.465 ± 0.01** (-20.24)
Malate dehydrogenase	0.044 ± 0.002	0.041 ± 0.005 (-6.82)	0.040 ± 0.002	0.023 ± 0.005** (-42.5)	0.042 ± 0.009	0.028 ± 0.006* (-33.33)

Values are represented as umol of formazan/mg protein/h.
Values are means, ± S.D. of six experiments.
Values in the parantheses are % changes over controls.
* Significantly different from control $p<0.01$.
** Significantly different from control $p<0.001$.

RESULTS AND DISCUSSION

In mice (*Mus boosuga*), toxicity symptoms were manifested initially as a weakening of leg muscles within 5 days of repeated HCP treatement. Prolonged treatment for 7 days lead to severe paralysis of hind quarters and the mice almost dragged their hind legs along the bottom of the cage. Other symptoms of intoxication included high respiratory rate and listlessness.

Results presented in Table 1 suggest that the mice were hyperglycaemic and hyperlactaemic after 3 and 7 days of treatment. The hepatic glycogen content was found to be progressively decreased at the three time periods. The hepatic lactate levels were elevated after 3 and 7 days treatment whereas the pyruvate levels were decreased at the three time periods. Both decreased pyruvate and increased lactate levels caused a pronounced rise of lactate/pyruvate (L/P) ratios of blood and liver. Hepatic NAD–lactate, succinate and malate dehydrogenases have showed diminished activities after 1, 3 and 7 days of HCP treatment (Table 3).

Repeated oral administration of sublethal dose of HCP (60 mg/kg/day) to the mouse produced alterations in the biochemical constituent of blood and liver (Table 1 & 2) and activity levels of hepatic LDH, SDH and MDH (Table 3). A rapid fall in liver glycogen content concomitantly with increased blood glucose levels suggests an enhanced hepatic glycogenolysis. Decreased glycogenesis or glyconeogenesis may also be attributed to the decreased glycogen content in liver (Elkeles and Tavill 1983). Despite progressive elevation in blood and liver lactate levels, hepatic NAD–LDH activity was decreased suggesting a reduced flux of lactate into neoglucogenic pathway and/or into Krebs citric acid cycle for oxidation. The concept of excess lactate assumes that the ratio of lactate/pyruvate (L/P) in the blood is approximated by the ratio of NAD/NADH in the cells and is reflective of tissue redox state. Because of relatively low pyruvate and high lactate contents, the blood and liver L/P ratios were elevated at the three time periods suggesting tissue hypoxia and enhanced rate of anaerobic glycolysis during hexachlorophene treatment.

Hepatic succinate and malate dehydrogenase activities showed consistent decrement during repeated HCP teretment suggesting a reduced oxidation of malate and succinate. Caldwell *et al.* (1972) have observed high affinity of rat liver mitochondria for hexachlorophene, with subsequent inhibition of the mitochondrial enzymes. The mechanism of HCP toxicity is not fully established. However binding of HCP to hepatic cell membranes (Mavier *et al.* 1976) and mitochondria, *in vitro* inhibition of succininoxidase, cytochrome oxidase (Gould *et al.* 1955) and decreased catalytic efficiency of various pyridine nucleotide dependent dehydrogenases in the presence HCP (Wang and Buhler 1978) suggest that similar sort of interactions may be involved in diminishing of hepatic SDH and MDH activities. Since these dehydrogenases catalyze oxidation–reduction reactions essential in controlling cellular metabolism, their diminished activities following HCP treatment could have adverse effects on the tissue energy state.

The results of the present study suggest the perturbation of the hepatic redox state and oxidative metabolism, together with the uncoupling of oxidative phosphorylation (Cammer and Moore 1972; Nakaue *et al.* 1972) result in a significant reduction in the cellular high energy phosphate reserves (Caldwell *et al.* 1972) during repeated HCP treatment. This perturbation may have a contributory role in the manifestation of hepatotoxicity by HCP (Prasad 1986).

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