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Effect of Heptachlor on Certain Aspects of Carbohydrate Metabolism in Swiss Albino Mice

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Modern trends in agriculture and forestry have seen toxicomania for organochlorine pesticides with little concern other than their efficacy against organisms. Agriculturists are after these organochlorines because of their persistance after pest kill. Accumulation of these compounds in animals could be seen with the ingestion of contaminated food, direct absorption from air and absorption through the integument of adsorbed material (Kerr and Vass, 1973). Heptachlor (1,4,5,6,8, 8-heptachloro - 3a, 4,7, 7a-tetra hydro-4,7-methano-1-H-indene) is widely used non systemic organochlorine insecticide in India. Heptachlor is a relatively stable one, it is used extensively for several years in soil, seed, and foliar treatment for insect control in agricultural crop fields (Swift, 1975). Because of its high potential toxicity still it has been used in India. Therefore, an attempt is made to explore the possible impact of heptachlor on carbohydrate metabolism in albino mice (Swiss albino) in the present investigation.

MATERIALS AND METHODS

Healthy adult albino mice (Swiss albino) of the same age group 26 ± 2g maintained in the mice colony at 27 ± 2°C in clean polypropylene cages with a normal photoperiod of 12 h light and 12 h darkness were used in the present investigation. They were fed with standard pellet diet and water *ad libidum*. Technical grade heptachlor (72%) was dissolved in acetone and distilled water to study acute and chronic effects. The pesticide was given to mice orally for *in vivo* studies. The animals were grouped into two batches. The first batch served as the control and second as experimental. The experimental animals were

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administered with heptachlor (16 mg/kg body weight) as single, double and multiple doses with 3 days of interval. After the stipulated time the animals were sacrificed and some aspects of carbohydrate metabolism were studied.

The following carbohydrate profiles were estimated in liver, muscle and kidney of albino mice. The total carbohydrates and glycogen were estimated according to Carrol et al. (1956) by Anthrone positive. Pyruvate content was estimated by using 2,4 DNP (2,4 Dinitrophenylhydrazine) by the method of Friedemann and Haugen (1942). Lactic acid was estimated spectrophotometrically by developing colour with para hydroxydiphenyl adopting the method of Barker and Summerson (1941), Lactate dehydrogenage (LDH) and Succinate dehydrogenage (SDH) activities were assayed by the method of Nachlas et al.(1960) by using INT (P iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride) in spectrophotometer. Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was estimated by the method of Lohr and Waller(1965) using NADP (Nicotinamide adenine dinucleotide phosphate). Phosphorylase activity was estimated spectrophotometrically by developing colour with ANSA (1-Amino-2-naphthol-4-sulphonic acid) by the method of Cori et al. (1955). Significance of the data was assessed through students "t" test.

RESULTS AND DISCUSSION

The results (Tables 1-3) show a significant and gradual decrease in total carbohydrates and glycogen of liver, muscle and kidney of mice as a function of the exposure period. It is found that the changes were pronounced more in liver compared to muscle and kidney. Since liver is considered as metabolic center where syntheses, transport and storage of carbohydrates occur and under stress the depletion of carbohydrates may be due to rapid utilization of energy to face the alteration caused by heptachlor.

Pyruvate and lactate levels can be used as a measurement to assess the aerobic and anaerobic environment of tissues of the organism under sublethal stress. The enhanced levels of lactic acid and depleted levels of pyruvate was observed in all tissues. Decrease in pyruvate indicates its role as a precursor for other products in metabolism like conversion to lactate or to form amino acids, lipids triglycerides (Sathya Prasad 1983). It can be assumed that hypoxic or anoxic condition brought about by stress may reduce the pyruvate content. Increase of lactate an end product of anaerobic condition and accumulation of lactic acid in tissues in the glycolytic segment induced by the toxicity

Table 1. Levels of total carbohydrate (mg/g wet weight), glycogen (mg/g wet weight), pyruvate (mg/g wet weight), lactate (mg/g wet weight), lactate dehydrogenase (μ moles of formazan formed/mg protein/h), succinate dehydrogenase (μ moles of formazan formed/mg protein/h). glucose-6-phosphate dehydrogenase (μ moles of formazan formed/mg protein/h) and phosphorylase (μ moles of inorganic phosphate formed/mg protein/h) in liver of mice under heptachlor intoxication.

Parameter	Control	Experimental		
		Single dose	Double dose	Multiple dose
Carbohydrates	14.84 ±2.55	9.24 ± 0.76	3.14 ±0.34	3.01 ±0.30
% change		(-37.74)	(-78.80)	(-79.10)
Glycogen	83.82 ±0.78	53.97 ±2.01	41.54 ±2.59	36.53 ±4.25
% change		(-35.61)	(-50.44)	(-56.41)
Pyruvate	31.50 ±1.95	38.34 ±7.26	24.40 ±1.29	22.38 ±2.97
% change		(-10.03) N.S.	(-22.53)	(-28.95)
Lactate	42.90 ±3.91	53.40 ±5.23	62.20 ±5.75	73.50 ±4.32
% change		(24.47)	(44.98)	(71.32)
Lactate dehydrogenase	2.23 ±0.39	3.77 ±0.73	5.35 ±0.65	6.98 ±0.81
% change		(69.05)	(139.90)	(213.00)
Succinate dehydrogenase	2.42 ±0.42	2.11 ±0.31	1.26 ±0.27	1.15 ±0.39
% change		(-12.80) N.S.	(-47.93)	(-52.47)
Glucose-6-phosphate dehydrogenase	5.97 ±0.98	6.23 ±1.62	8.76 ±1.37	10.79 ±1.97
% change		(4.35) N.S.	(46.39)	(80.73)
Phosphorylase	2.19 ±0.40	2.48 ±0.42	3.62 ±0.68	5.00 ±0.70
% change		(13.24) N.S.	(65.20)	(128.60)

Values are mean ± S.D. of six individual observations.
All differences are significant at 0.05 level.
N.S. = Non significant.

Table 2. Levels of total carbohydrate (mg/g wet weight), glycogen (mg/g wet weight), Pyruvate (mg/g wet weight), lactate (mg/g wet weight), lactate dehydrogenase (µ moles of formazan formed/mg protein/h), succinate dehydrogenase (µ moles of formazan formed/mg protein/h), glucose-6-phosphate dehydrogenase (µ moles of formazan formed/mg protein/h) and phosphorylase (µ moles of inorganic phosphate formed/mg protein/h) in muscle of mice under heptachlor intoxication.

Parameter	Control	Experimental		
		Single dose	Double dose	Multiple dose
Carbohydrates	10.38 ±1.38	4.76 ±0.44	4.12 ±0.41	2.57 ±0.38
% change		(-54.14)	(-60.30)	(-75.20)
Glycogen	35.77 ±0.86	32.19 ±0.031	28.98 ±1.11	25.98 ±0.41
% change		(-10.00)	(-18.98)	(-27.37
Pyruvate	22.72 ±1.03	20.17 ±3.13	12.15 ±1.75	9.57 ±2.12
% change		(-11.22) N.S.	(-46.52)	(-57.87)
Lactate	49.70 ±4.34	52.30 ±4.01	60.47 ±3.39	71.32 ±5.92
% change		(5.23) N.S.	(21.67)	(43.50)
Lactate dehydrogenase	1.39 ±0.35	1.79 ±0.59	2.59 ±0.69	4.36 ±0.72
% change		(28.77) N.S.	(86.33)	(213.60)
Succinate dehydrogenase	1.37 ±0.39	1.22 ±0.37	1.19 ±0.23	0.98 ±0.19
% change		(-10.95) N.S.	(-13.14)	(-28.47)
Glucose-6-phosphate dehydrogenase	3.11 ±0.23	4.39 ±0.21	5.41 ±0.62	5.62 ±0.51
% change		(41.16)	(73.95)	(80.70)
Phosphorylase	2.12 ±0.38	2.35 ±0.67	4.10 ±0.68	4.62 ±0.68
% change		(10.84)	(93.39)	(117.90)

Values are mean ± S.D. of six individual observations.
All differences are significant at 0.05 level.
N.S. = Non significant.

Table 3. Levels of total carbohydrate (mg/g wet weight), glycogen (mg/g wet weight), pyruvate (mg/g wet weight), lactate (mg/g wet weight), lactate dehydrogenase (µ moles of formazan formed/mg protein/h), succinate dehydrogenase (µ moles of formazan formed/mg protein/h), glucose-6-phosphate dehydrogenase (µ moles of formazan formed/mg protein/h) and phosphorylase (µ moles of inorganic phosphate/mg protein/h) in kidney of mice under heptachlor intoxication.

Parameter	Control	Experimental		
		Single dose	Double dose	Multiple dose
Carbohydrates	5.01 ±0.54	4.82 ±0.45	3.11 ±0.32	2.41 ±0.30
% change		(-3.79) N.S.	(-37.90)	(-51.90)
Glycogen	16.11 ±0.51	15.74 ±0.55	14.39 ±0.89	13.04 ±0.99
% change		(-2.30) N.S.	(-10.68)	(-19.05)
Pyruvate	20.58 ±1.52	17.63 ±1.26	17.96 ±1.01	15.58 ±1.42
% change		(-14.37)	(-12.73)	(-24.29)
Lactate	45.20 ±2.92	49.60 ±2.61	55.32 ±2.45	65.39 ±4.32
% change		(9.73)	(22.38)	(44.46)
Lactate dehydrogenase	1.17 ±0.29	1.59 ±0.47	2.28 ±0.57	3.00 ±0.63
% change		(35.89)	(94.87)	(156.4)
Succinate dehydrogenase	1.21 ±0.27	1.19 ±0.34	1.09 ±0.15	0.79 ±0.13
% change		(-1.65) N.S.	(-9.92) N.S.	(-34.71)
Glucose-6-phosphate dehydrogenase	2.19 ±0.37	2.98 ±0.39	3.79 ±0.47	4.60 ±0.76
% change		(36.07)	(73.05)	(110.00)
Phosphorylase	1.39 ±0.17	1.98 ±0.29	2.43 ±0.39	2.89 ±0.40
% change		(42.40)	(74.82)	(107.90)

Values are mean ± S.D. six individual observations.
All differences are significant at 0.05 level.
N.S. = Non significant.

and the tendency of shift from aerobic to anaerobic pathways to meet energy demand of metabolic activity (Abston and Yarbrough 1976). LDH levels which indicate the energy demands are met by anaerobic respiration through increase in LDH activity. Pesticides are known to cause cellular disintegration, mitochondrial damage and anaerobiasis. Increased permeability of cells and necrosis are usually characterised by rise in LDH activity (Radhaiah 1985). The demolishment of SDH activity levels in the tissues suggests the inhibition of oxidative metabolism at mitochondrial level, probably due to change in ultrastructure and morphology of mitochondria (Miroslaw et al.1973). It is likely that the tissue levels lead to the damage of mitochondrial integrity (Graham and Hasen 1972).

The increased levels of G-6-PDH content may be considered as a compensation towards diminished carbohydrate reserves to meet the energy demands. The increase in G-6-PDH corresponds with elevated Hexose mono phosphate shunt is to produce Nicotinamide adenine dinucleotide phosphate hydrogen which may act as alternate source to overcome energy crisis. The increased oxidation of glucose through switched over HMP shunt by G-6-PDH is due to the prevalent of anaerobiasis. The maximum increase of G-6-PDH levels under heptachlor stress may result in production of more pentoses and NADP for synthesis and detoxification purposes, possibly to mitigate stress and this can be considered as a biochemical adaptive phenomenon during pesticidal stress (Kohli et al. 1975). The consistent phosphorylase activity observed in the present investigation supported the breakdown or glycogen to glucose-6-phosphate. Since liver is considered to be the metabolic center for synthesis, transport and storage of carbohydrate, it experienced maximum changes in carbohydrate profiles during stress condition. The interaction of muscle and kidney with the toxic molecules is comparatively lesser than that of liver. Thus the alterations in carbohydrate profiles as a function of heptachlor toxicity is to tide over the prevailed energy crisis.

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