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Changes in the Protein Metabolism in Liver and Kidney of *Mus booduga* Gray after Oral BHC Feeding

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Recent years have seen phenomenal increases in the use of pesticides to control various types of pests to increase food supply. Among them the organochlorine (OC) group of insecticides are widely used in India because of their cheapness and long persistency. The technical grade Benzenehexachloride (BHC - 1,2,3,4,5,6-Hexachlorocyclohexane) is a mixture of several stereo isomers (Matsumura 1975) and it has low acute toxicity but high chronic toxicity to animals mainly due to the accumulation and slow degradation of β-isomer in animal tissues (Wester et al 1985). Since the derrangement in tissue protein degradation is reflected by changes in protein composition (Murthy and Priyamvada Devi 1982), later may be considered in assessing the protein metabolic perturberations during toxic stress. So, the present work is aimed to study the effect of BHC on the protein metabolism in the mice, Mus booduga.

MATERIALS AND METHODS

Adult mice, Mus booduga weighing 10-12 g were acclimated to laboratory conditions for 10 days and had free access to food and water before using them for experimentation. Technical grade BHC (1,2,3,4, 5,6-Hexachlorocylohexane) obtained from MICO farm chemicals, Madras, India was dissolved in corn oil and was administered orally for 1, 5 and 15 days at a dose of 50 mg/kg b.w./day. Controls were fed with isovolumetric amount of corn oil (0.02 ml). After the stipulated time the tissues were isolated and chilled in ice box and used for the estimation of metabolites and enzymes. 5% homogenates were prepared in 0.25 M sucrose solution for proteins, Asparate aminotransferase (AAT), Alanine aminotransferase (AlAT) and Gultamate dehydrogenase (GDH) and in ice-cold distilled water for protease. Homogenates were centrifuged at 2500 g for 10 mins at 4°C to remove cell debris. The clear cell-free extract was used for enzyme assays. 10% homogenates were prepared in distilled water for ammonia and in 15% perchloric acid for urea. Protein content was determined with Folin Phenol reagent (Lowry et al 1951) using Bovine serum albumin as standard. Proteins were precipitated with 10% Trichloroacetic acid (TCA) and the protein free supernatant was processed

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for Free Amino Acids (FAA) estimation by using Ninhydrin reagent (Moore and Stein 1954) and Tyrosine as standard. Ammonia was estimated (Bergmeyer 1965) using Ammonium chloride as standard and the Urea levels were studied with diacetyl monaxime method (Natelson 1971) using urea as standard.

Protease activity was determined by the amount of tyrosine formed/h/mg protein at 37°C incubation by the method of Moore and Stein (1954). The reaction mixture contains 100 μ moles of phosphate buffer (pH 7.2) and 12 mg of denatured protein. AAT and AlAT activities were assayed by the amount of pyruvate formed/h/mg protein at 37°C incubation (Reitman and Frankel 1957). The incubation mixture of AAT contains 100 μ moles of phosphate buffer (pH 7.4), 50 μ moles of L-aspartic acid (pH 7.4) and 2 μ moles of α-ketoglutaric acid. For AlAT incubation steps followed are the same as described for AAT but the substrate used was DL-alanine (2 μ moles). GDH was assayed by the method of Lee and Lardy (1965). The reaction mixture contains 100 μ moles of phoshate buffer (pH 7.4), 40 μ moles of sodium glutamate, 0.1 μ mole of NAD and 4 μ moles of INT. The activity was expressed in μ moles of formazan formed/h/mg protein. The standard graphs were prepared with sodium pyruvate for AAT and AlAT and with formazan for GDH. The mean values of control and BHC treated mice of all the parameters mentioned above were subjected to statistical treatment using 't' test of significance (Bailey 1965).

RESULTS AND DISCUSSION

The soluble, structural and total proteins showed a decrease in kidney and liver tissues of BHC treated mice (Tables 1 & 2) with varying levels of statistical significance. The decrease in proteins may be due to their degradation and also due to possible utilization of these compounds for metabolic purposes (Murthy and Priyamvada Devi 1982) and involvement in osmoregulation (Moorthy et al 1984). The FAA are found to play a vital role in synthesis of enzymes and hormones and their increased levels in liver and kidney indicate stepped up proteolysis, fixation of ammonia and keto acids resulting in amino acid formation (Kabeer Ahmed et al 1978). Depletion of tissue proteins of animals exposed to pesticides has been reported earlier (Kabeer Ahmed et al 1978; Murthy and Priyamvada Devi 1982).

The transaminase levels were increased in both the tissues (liver and kidney) of BHC treated mice (Tables 1 & 2).

The aminotransferases serve as a strategic link between carbohydrate and protein metabolism under environmental stress (Knox and Greengard 1965). Increases in AAT and AlAT levels indicate that there is an active transamination of amino acids and operation of keto acids which are probably fed into the TCA cycle for oxidation. The increase in the activities of hepatic aminotransferases in the present study is in agreement with earlier reports demonstrating consistent increases in these activities under conditions of enhanced gluconeogenesis (Knox and Greengard 1965) and increased glucocorticoid activity during dieldrin toxicity (Bhatia et al 1970). Dikshith et al (1978) have reported similar effects on the liver

Table 1. Metabolic changes in the liver of Mus booduga* treated with 50 mg/kg b.w./day BHC

Parameter	Control	Sublethal treatment (days)		
		1	5	15
Total proteins (mg/g wet wt.)	382.8 ±27.3	364.1±24.5[d] (-4.88)	337.1±27.5[c] (-11.69)	312.4±23.1[b] (-18.39)
Soluble proteins (mg/g wet wt.)	210.2 ±14.7	200.4±15.9[d] (-4.66)	188.2±10.6[c] (-10.47)	179.2±14.0[b] (-14.75)
Structural proteins (mg/g wet wt.)	171.1 ±11.3	164.1±10.6[d] (-4.09)	149.1±14.1[c] (-12.86)	133.4±13.7[a] (-22.03)
FAA (μ moles of tyrosine/g wet wt.)	22.2 ± 3.8	24.4±4.23[d] (+9.91)	26.3±3.96[d] (+18.5)	28.5±3.93[c] (+28.4)
Protease (μ moles of tyrosine/h/mg protein)	0.62 ± 0.17	0.71±0.05[d] (+14.5)	0.81±0.05[c] (+30.6)	0.89±0.04[b] (+43.5)
GDH (μ moles of formazan formed/ h/mg protein)	0.23 ± 0.03	0.31±0.04[b] (+34.8)	0.38±0.04[a] (+65.2)	0.43±0.15[b] (+86.9)
AAT (μ moles of pyruvate formed/ h/mg protein)	1.22 ± 0.18	1.62±0.17[b] (+32.8)	2.13±0.34[a] (+74.6)	2.34±0.25[a] (+91.8)
AlAT (μ moles of pyruvate formed/ h/mg protein)	3.85 ± 0.29	4.43±1.28[d] (+15.1)	5.14±1.15[c] (+33.5)	5.37±0.76[b] (+37.5)
Ammonia (μ moles of ammonia/g wet wt.)	0.79 ± 0.06	0.90±0.06[c] (+13.9)	1.04±0.31[d] (+31.6)	0.98±0.07[a] (+24.1)
Urea (μ moles of urea/g wet wt.)	3.36 ± 0.55	3.85±0.29[d] (+14.6)	4.18±0.77[d] (+24.4)	3.62±0.25[d] (+7.74)

* Values are mean ± SD (n=6); Values in the parenthesis indicate per cent change over control.
FAA, Free amino acids; GDH, Glutamate dehydrogenase;
AAT, Asparate aminotransferase; AlAT, Alanine aminotransferase.
[a]P < 0.001 [b]P < 0.01 [c]P < 0.05 [d]Not significant.

transminase levels of guinea pigs treated with lindane (100 mg/kg b.w./day) for 15 days. Enhanced levels of transaminases were also observed in Anabas testudineus exposed to lindane (Bhakthavatsalam and Srinivasa Reddy 1982).

Table 2. Metabolic changes in the kidney of Mus booduga* treated with 50 mg/kg b.w./day BHC

Parameter	Control	Sublethal treatment (days)		
		1	5	15
Total proteins (mg/g wet wt.)	342.8 ±28.2	322.1±26.6[d] (-6.04)	307.2±29.5[d] (-10.38)	294.1±21.3[c] (-14.21)
Soluble proteins (mg/g wet wt.)	185.3 ±12.9	171.2±14.2[d] (-7.61)	160.3±14.4[c] (-13.49)	148.2±12.8[b] (-20.02)
Structural proteins (mg/g wet wt.)	157.3 ±12.1	151.6±14.1[d] (-3.62)	147.2±12.2[d] (-6.42)	146.1±12.4[d] (-7.12)
FAA (µ moles of tyrosine/g wet wt.)	18.6 ±4.01	20.3±3.66[d] (+9.14)	21.4±4.04[d] (+15.1)	22.6±4.58[d] (+21.5)
Protease (µ moles of tyrosine/h/mg protein)	0.45 ±0.14	0.55±0.04[d] (+22.2)	0.61±0.05[c] (+35.6)	0.68±0.05[b] (+51.1)
GDH (µ moles of formazan formed/ h/mg protein)	0.30 ±0.04	0.35±0.03[c] (+16.7)	0.38±0.16[d] (+26.7)	0.45±0.15[c] (+50.0)
AAT (µ moles of pyruvate formed/ h/mg protein)	1.62 ±0.42	1.90±0.37[d] (+17.3)	2.18±0.33[c] (+34.6)	2.63±0.32[b] (+62.3)
AlAT (µ moles of pyruvate formed/ h/mg protein)	3.62 ±0.25	4.46±1.00[d] (+23.2)	4.91±0.93[c] (+35.6)	5.63±1.32[b] (+55.5)
Ammonia (µ moles of ammonia/g wet wt.)	1.12 ±0.26	1.27±0.38[d] (+13.4)	1.56±0.32[c] (+39.3)	1.75±0.43[c] (+56.3)
Urea (µ moles of urea/g wet wt.)	0.86 ±0.07	1.07±0.29[d] (+24.4)	1.28±0.35[c] (+48.8)	1.43±0.43[b] (+66.3)

* Values are mean ± SD (n=6); Values in the parenthesis indicate per cent change over control.
FAA, Free amino acids; GDH, Glutamate dehydrogenase;
AAT, Asparate aminotransferase; AlAT, Alanine aminotransferase.
[a]P < 0.001 [b]P < 0.01 [c]P < 0.05 [d]Not significant.

Glutamate dehydrogenase activity increased in both the tissues of BHC treated mice (Tables 1 & 2) suggesting effective operation of oxidative deamination process under toxic impact. This is also corroborated by the observed increased levels of ammonia in both the tissues (Tables 1 & 2). Such increased ammonia levels might also be due to some

other deaminative processes since the activity levels of AMP deaminases and nucleotide deaminases were enhanced during pesticide exposure (Murthy et al 1985). The urea content also increased in both the tissues of BHC treated mice. From the data it is evident that excess of liver ammonia is converted to urea, which means that the liver tissue has accelrated urea synthesis to detoxify the excess of ammonia either produced by the liver per se or being transported from other tissues.

Liver and kidney tissues were reported to be the sites of degradation and detoxificiation of OC insecticides (Devi et al 1981) and the biochemical effects recorded seem to be the result of greater stress. In general, the changes induced by BHC were more in liver than kidney. Death of mice in acute studies to high dose of toxicants may be due to their direct action. However, significant biochemical changes induced by pesticides may be more hazardous and could reduce the growth rate, fecundity and effect the ability to assimilate food. The pesticide induced chronic biochemical alterations are poorly known and need to be investigated more extensively.

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