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Effect of Insecticides on *Achaea janata* Linn. II. Haemolymph-free Amino Acids

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A comparison was made on the effect of four different insecticides on the haemolymph-free amino acids. A total of nineteen amino acids were detected by amino acid autoanalyzer. Treatment with pp'-DDT resulted in the depletion of amino acids like threonine/glutamine/asparagine, proline, alanine, valine and histidine. Heptachlor caused reduction of threonine/glutamine/asparagine, proline, glutamic acid, alanine and tyrosine, whereas malathion resulted in increase in concentration of most of the amino acids. However, carbaryl, in general, depleted most of the amino acids. Insecticidal stress, irrespective of the type of insecticide used, resulted in tremendous increase in an unidentified compound. Possible role of these changes under insecticidal stress has been discussed.

Key Words : Insecticides, Haemolymph, Free amino acids, *Achaea janata*

Introduction

Insect haemolymph is the richest source of free nitrogen in the form of amino acids. These free amino acids are of great importance for both growth and differentiation (Gilmour 1965 and Chen 1971). Amino acids enter into different metabolic pathways at various points and participate in physiological activities. Unlike mammalian blood, insect haemolymph is in direct contact with the tissues. Hence, any disturbance in the body from any source is reflected by changes in the haemolymph.

Various abnormalities are known to occur when insects are exposed to xenobiotics. In our earlier studies (Ramdev & Rao 1986), we reported that under the insecticidal stress, insects lose body weight and haemolymph volume, coupled with alteration in cations concentrations. There is no information on the effect of insecticides on amino acids of haemolymph, especially in holometabolus insects. In continuation of our work on the effect of insecticides on nutritional profile (Ramdev & Rao 1980) and haemolymph constituents (Ramdev & Rao 1986), the present work is focussed on the effects of pp'-DDT, heptachlor, malathion and carbaryl on free amino acids of *Achaea janata*.

Materials and Methods

Larvae of the castor semilooper, *Achaea janata*, were maintained in the laboratory on castor leaves at 27° ± 1°C and 70% relative humidity (Ramdev & Rao 1979). For the present investigation, 36 hr old 5th instar larvae were used.

Toxicity and application of insecticides: Four insecticides belonging to chlorinated hydrocarbon, cyclodiene, organophosphate and carbamate groups, were selected and these were pure pp'-DDT [1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane], heptachlor (72%) (1, 4, 5, 6, 7, 10, 10-heptachloro-4, 7, 8, 9-tetrahydro-4, 7-methyleneindane), malathion (95%) [0, 0-dimethyl S-(1, 2-dicarboethoxy ethyl phosphorodithioate] and carbaryl (99%) (1-naphthyl N-methylcarbamate), respectively. Details on the method of application, toxicity of insecticides and determination of LD_{50} have been discussed elsewhere (Ramdev &

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Rao 1986). LD_{50} for pp'-DDT, heptachlor, malathion and carbaryl were: 175.33, 204.33, 81.93 and 17.46 μg/g body weight respectively.

LD_{50} dose of respective insecticides was used for the study of insecticidal effects on free amino acids. This facilitated to study the effects for 60 hr after treatment and thus covered the period of this instar. Insects treated with pure acetone served as controls.

Sampling time: Haemolymph samples, both from treated and control larvae, were collected at 0.5, 6, 12, 24, 36, 48 and 60 hr after the treatment. The method of collection, processing of haemolymph and estimation of amino acids by analyser, have been discussed earlier (Ramdev & Rao 1984).

The data presented consist of only per cent variations of treatment from the control. Thus, any increase or decrease in concentration of an amino acid in treatments is always with refrence to its corresponding control at that point of observation. The data were also subjected to ANOVA taking into consideration the different time intervals of poisoning of each insecticides as a block.

Results

Both control and treatment showed the maximum of 19 free amino acid peaks, including one unidentified peak. The comparative data on the effect of insecticides on free amino acids are presented in figures 1a–g.

pp'-DDT: Threonine-glutamine-asparagine group was the most affected one by DDT treatment. The concentration of this group was lower to control throughout the period of poisoning. Glutamic acid level was also reduced after treatment except at 0.5 and 36 hr. A reduction up to 88.5% was observed up to 12 hr of poisoning. Proline showed a reduction up to 24 hr of poisoning followed by an increase at 36 and 48 hr. Leucine concentration also fluctuated similar to proline after the treatment. Glycine was also affected and was lower to control at all stages of poisoning except at 12 hr. Alanine and valine also presented a similar picture. Histidine decreased during early phase of poisoning (up to 36 hr) with a 4-fold increase at 48 hr. However, the concentration of unidentified compound was throughout higher in treatments over control.

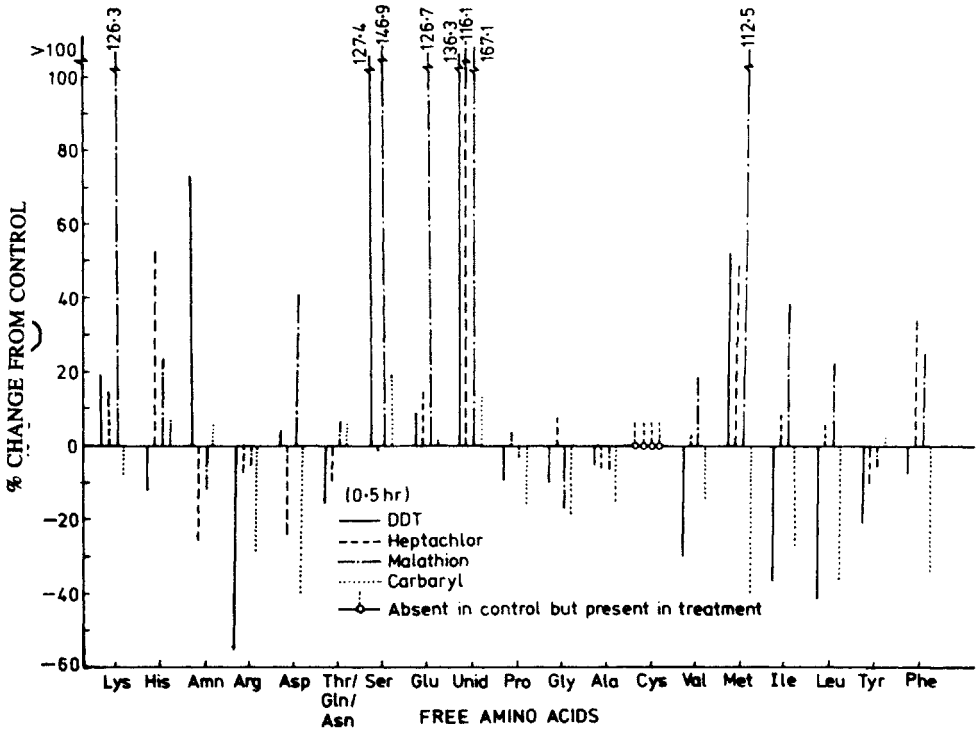
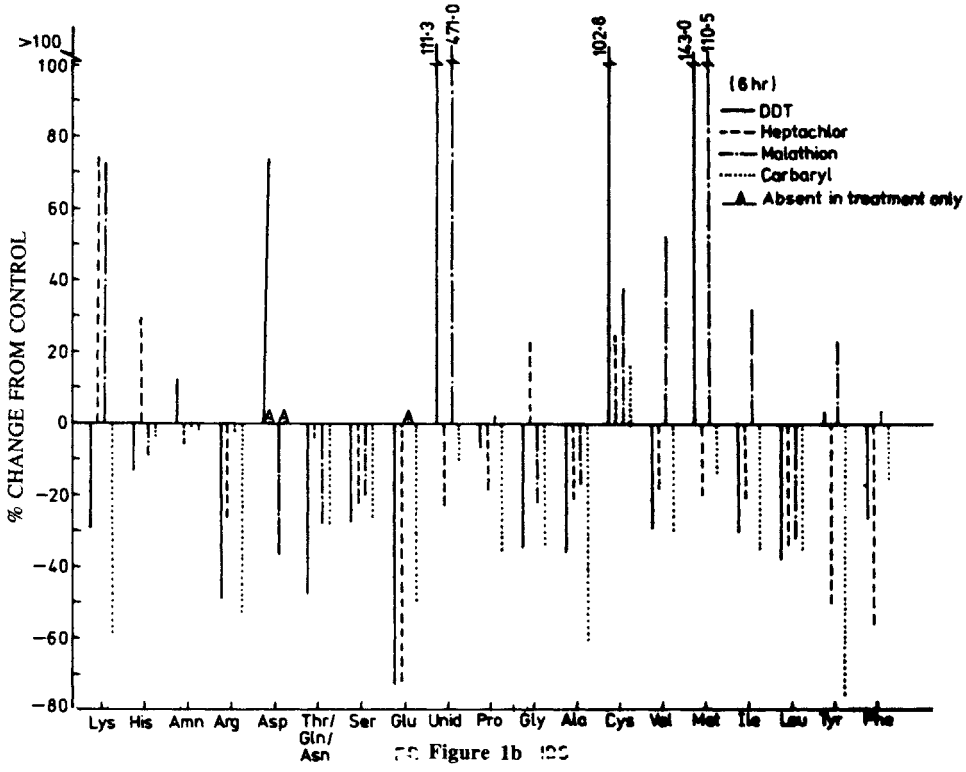


Figure 1a

Figure 1 a–g Effect of insecticides on haemolymph-free amino acids of 5th instar *Achaea janata*. **a**, 0.5 hr; **b**, 6.0 hr; **c**, 12.0 hr; **d**, 24.0 hr; **e**, 36.0 hr; **f**, 48.0 hr; **g**, 60.0 hr


% CHANGE FROM CONTROL
>100
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80
60
40
20
0
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111·3
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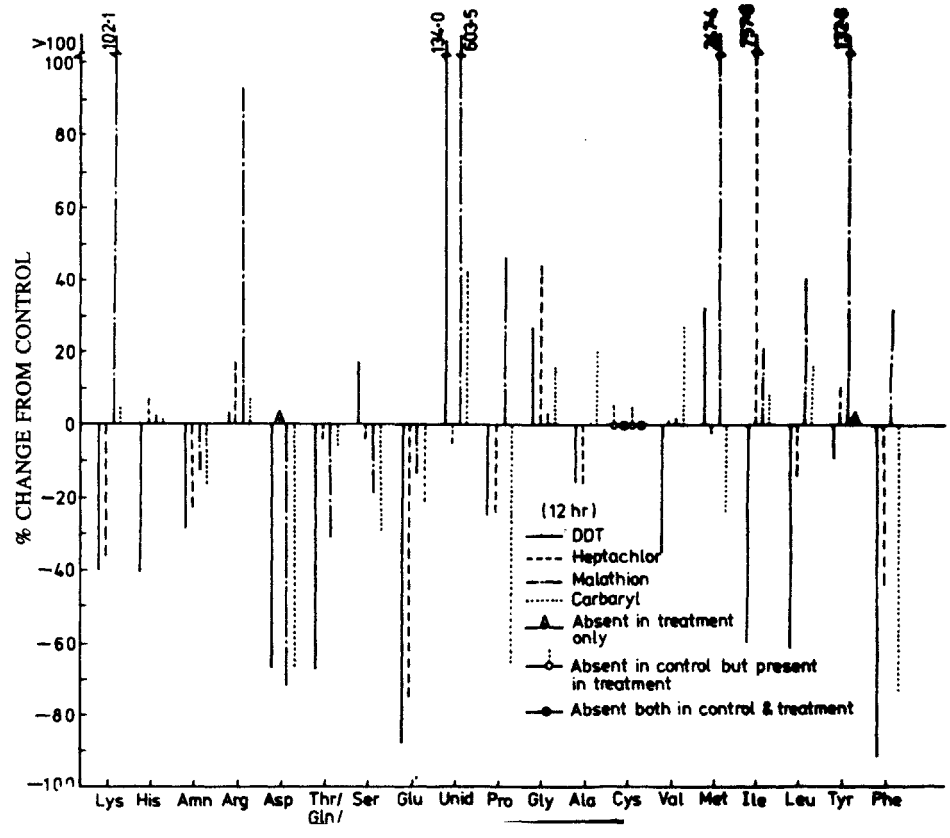
143·0
110·5
(6 hr)
DDT
Heptachlor
Malathion
Carbaryl
Absent in treatment only
Lys His Amn Arg Asp Thr/ Gln/ Asn Ser Glu Unid Pro Gly Ala Cys Val Met Ile Leu Tyr Phe

Figure 1b


% CHANGE FROM CONTROL
>100
100
80
60
40
20
0
−20
−40
−60
−80
−100
102·1
134·0
603·5
(12 hr)
DDT
Heptachlor
Malathion
Carbaryl
Absent in treatment only
Absent in control but present in treatment
Absent both in control & treatment
Lys His Amn Arg Asp Thr/ Gln/ Ser Glu Unid Pro Gly Ala Cys Val Met Ile Leu Tyr Phe

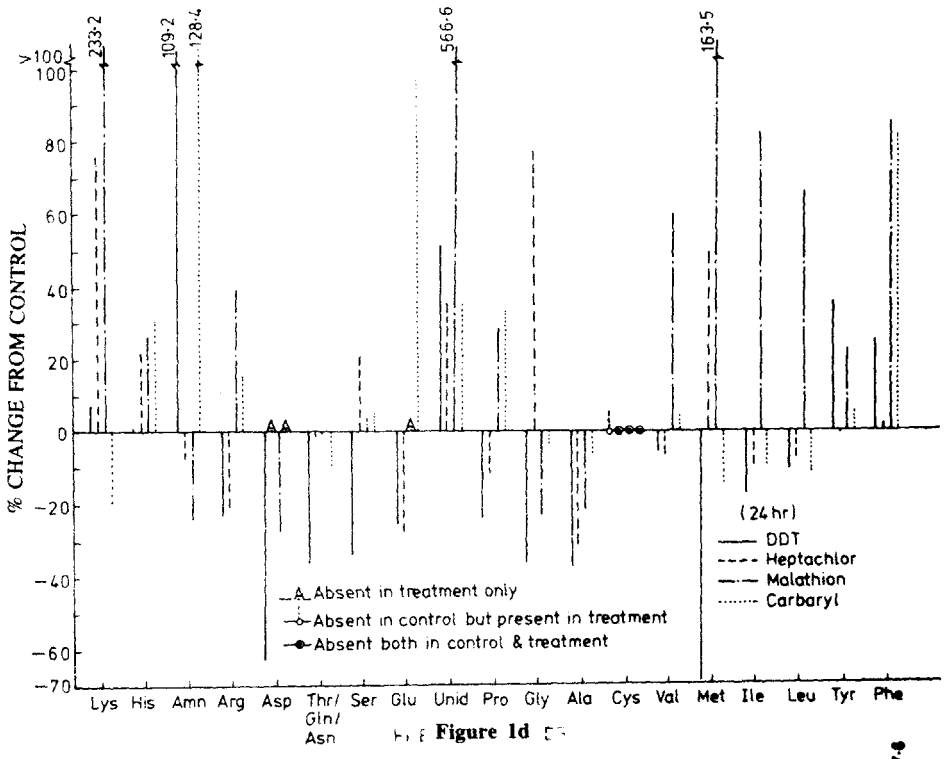


Figure 1d

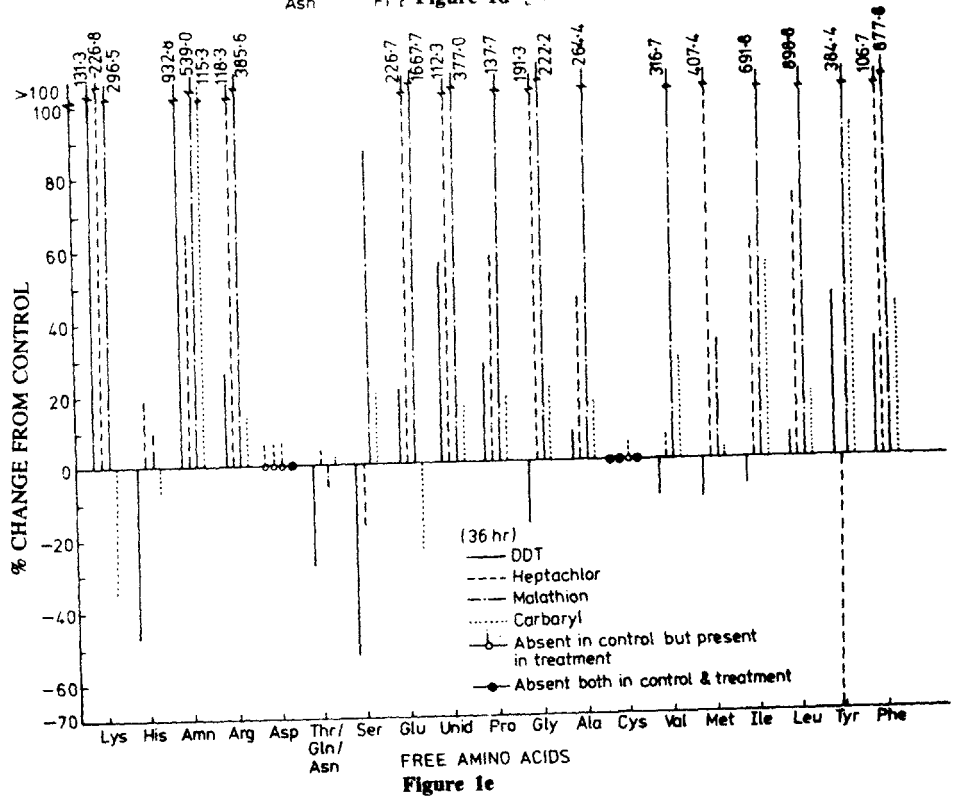


Figure 1e

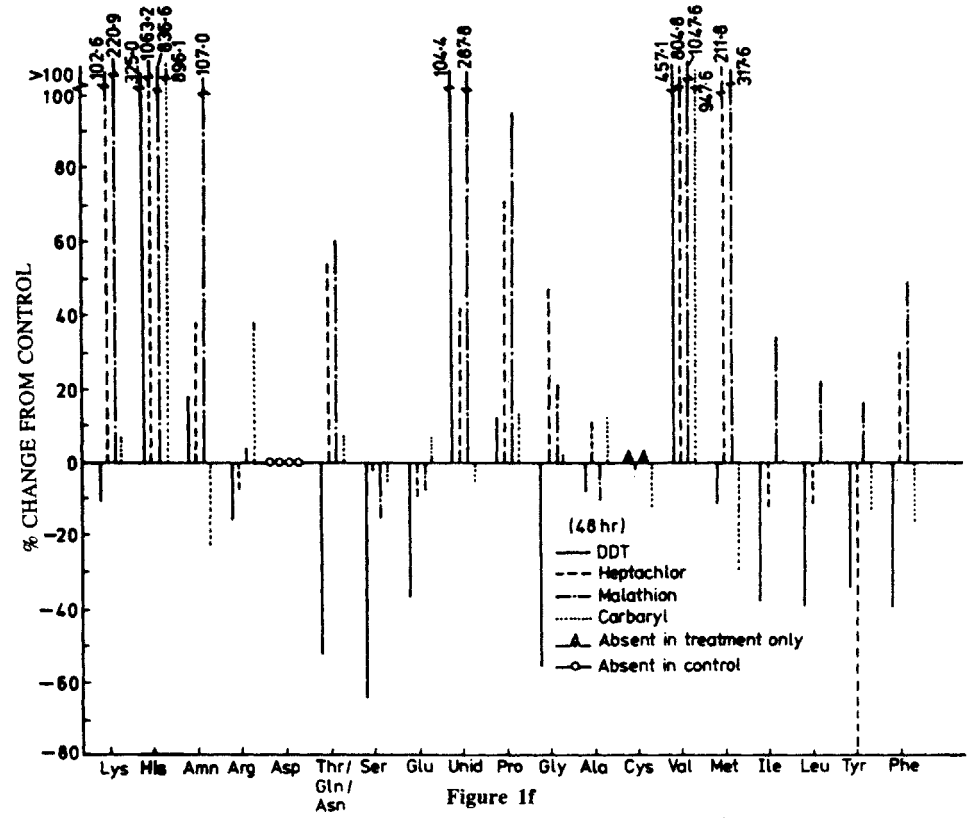


Figure 1f

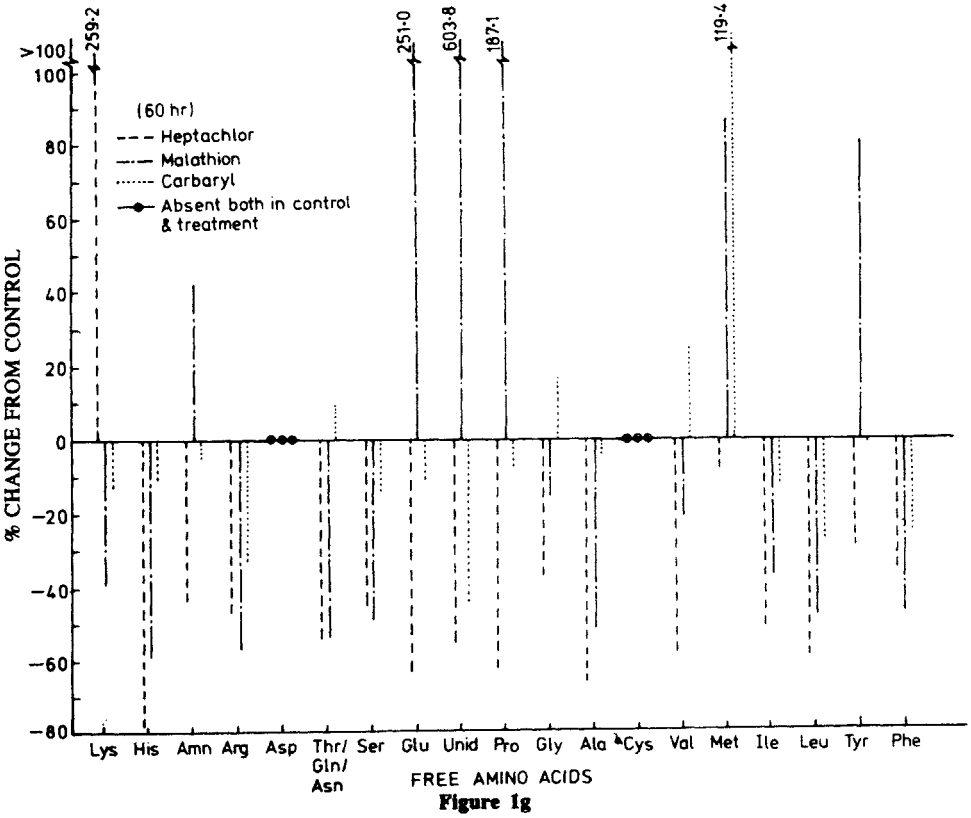


Figure 1g

Heptachlor: Heptachlor treatment resulted in the reduction of Thr/Gln/Asn concentration during first 24 hr with subsequent increase at 36 and 48 hr. At the later stage of poisoning (60 hr), its concentration again decreased below to that of control. Alanine behaved similar to Thr/Gln/Asn group. Glutamic acid concentration in treatment was more than in control at early stage of poisoning (0.5 hr). Thereafter there was reduction in its concentration at all stages of poisoning except at 36 hr. Heptachlor caused an increase in proline concentration at early stage of poisoning (0.5 hr) followed by reduction during next 24 hr. Proline concentration sharply increased again at 36 and 48 hr, the maximum (71.8%) being at 48 hr. Finally, there was reduction (60.3%) once again at 60 hr after treatment. Tyrosine decreased in treatment throughout except at 12 hr. However, glycine concentration increased throughout the poisoning period except at 60 hr.

Malathion: Malathion treatment, in general, increased the concentration of most of the amino acids during various stages of poisoning. Maximum increase was observed at 36 hr after treatment. Increase in glutamic acid was as high as 17-fold at 36 hr.

Proline concentration decreased at the begining (0.5 hr) but subsequently increased which continued up to 60 hr. Tyrosine behaved similar to proline. Except at 36 hr, lower concentration of alanine was observed throughout the poisoning period. At 36 hr, concentration was about 4 times higher than in control. However, no difference in concentration was found between treatment and control at 12 hr. Malathion effect on methionine and unidentified compound was tremendous. Throughout the period of poisoning, concentrations of these compounds were much higher to control.

Carbaryl: Carbaryl, in general, depleted most of the amino acids. It decreased lysine concentration as compared to control throughout the period of poisoning except at 12 and 48 hr. In treatments, Thr/Gln/Asn showed increase over control within 30 min of poisoning whereas from 6 hr reduction was noticed which continued up to 24 hr and this was followed by an increase. Proline concentration depleted during early phase of poisoning (up to 12 hr) improved at late stages of poisoning. Carbaryl caused reduction in tyrosine concentration at 6 hr and its complete disappearance at 12 hr. Concentration of unidentified compound increased during the first 36 hr and decreased subsequently.

Results on analysis of variance are presented in table 1. The results show that out of 19 free amino acids, only 12 show significant differences among the treatments. Seven amino acids which were not affected by treatments are Arg, Asp, Ser, Glu, Ile and Leu.

Discussion

Winteringham (1958) suggested that the haemolymph amino acids provide a soluble and readily available substrate reserve for the tricarboxylic acid (TCA) cycle since many amino acids are readily degraded through this cycle to acetyl CoA, pyruvate or TCA cycle intermediates (Chefurka 1965). According to Mansingh (1965a), level of free amino acids in the intoxicated insect is influenced by the dose of insecticide used for poisoning (sublethal or lethal dose) and the time of evaluation of toxic effects. Since the effects observed in the present studies pertain to a single dose, the differences observed for a particular insecticide could only be attributed to the time of evaluation. Depletion of various amino acids after insecticidal treatment was as follows:

DDT: Threonine/glutamine/asparagine, proline, glutamic acid, alanine, valine and histidine.

Heptachlor: Threonine/glutmine/asparagine, proline, glutamine acid, alanine and tyrosine.

Malathion: Threonine/glutamine/asparagine, alanine and glycine.

Carbaryl: In general, depleted most of the amino acids.

In case of carbaryl, since the visual symptoms of poisoning were observed between 0–6 hr, maximum effect of this treatment on amino acids was during this period. Almost all amino acids showed reduction in their concentration during first 6 hr of poisoning.

Depletion in amino acids could result due to their utilization either for protein synthesis or for energy purpose. Insecticidal stress leads to greater metabolic activity with its associated high energy requirement in treated insects. Utilization of amino acids for energy purpose under such conditions was reported by Mansingh (1965a). He showed increased utilization of labelled amino acid by the poisoned cockroaches. Agosin et al. (1961) gave evidence for the inhibition of certain enzymes in DDT-poisoned *Triatoma infestans*, and increased oxygen consumption in malathion treatment was reported by Harvey and Brown (1951). It could be that depletion of amino acids in the present studies may be due to their utilization through TCA cycle. Reduction of glycine in malathion-treated insects may be due to its entry into the TCA cycle via pyruvate which then gets catabolized to CO_2 (Mansingh 1965b), while that of aspartic acid reduction could be accounted for its active utilization in transamination reaction during high neuromuscular activity. Amongst the amino acids, proline was the most affected by carbaryl, DDT and heptachlor treatments. The results are in agreement with the work of Corrigan and Kearns (1958, 1963)

Table 1. *Effect of insecticides on haemolymph free amino acids (μmoles/ml) of* Achaea janata *Linn.*

Treatment	Lys	His	Amm	Thr/Gln Asn	Unident	Pro	Gly	Cys	Val	Met	Tyr	Phe
CONTROL	2.66	6.85	4.74	11.88	3.6	13.52	3.89	0.12	2.43	0.87	1.55	0.89
pp'-DDT	2.84	5.05	10.59*	6.95*	7.2	12.38	2.91	0.27	1.84	1.26	1.55	0.59
HEPTACHLOR	4.69*	8.16	4.49	11.63	4.91	13.23	5.89*	0.2	2.44	1.28	1.07	0.85
MALATHION	6.27*	7.46	7.67	10.82	19.02*	20.21*	4.52	0.74*	4.17*	2.23*	2.66*	1.64*
CARBARYL	2.21	3.41*	5.8	11.4	3.8	12.08	3.69	0.18	2.78	0.71	1.3	0.74
C.D.	1.78	2.46	4.97	2.00	3.89	6.67	1.76	0.6	1.28	0.72	0.77	0.73

*Significant at 5%.

who postulated that poisoning produced a shift in the demand for oxidisable carbon to a carbon pool other than that provided by anaerobic glycolysis. Hence, proline was probably oxidised to CO_2, under insecticidal stress. Increase or decrease in concentrations of proline, glutamate and glutamine in DDT treated insects appears to be interrelated. Increase in proline during last stages of poisoning was probably due to greater accumulation of glutamate as a result of which conversion of glutamate to proline occurred. Conversion of glutamate to proline and vice versa is already known in insects (Mansingh 1965, Corrigan & Kearns 1963).

Increase in glutamine concentration (Thr/Gln/Asn) at 0.5 hr after malathion treatment suggested that the glutamate formed from proline was reduced by glutamate dehydrogenase to ammonia which was then utilized in the synthesis of glutamine. Increase in the concentrations of amino acids at later stage of poisoning is not clear. This general increase was probably due to protein catabolism or leakage of free amino acids from tissues.

In case of carbaryl, increase in amino acid concentration after 24 hr could be due either to greater protein catabolism or lesser utilization of amino acid. The concentration of methionine, a sulphur-containing amino acid decreased in contrast to increase in DDT, heptachlor and malathion-treated larvae. Perhaps, there was greater demand for sulphur by the insect body under carbamate poisoning.

Increase in the concentration of unidentified compound after insecticidal treatment is interesting. Six-fold increase was observed after malathion treatment. It was reported (Beament 1985, Sternburg et al. 1959) that under stress (physical or chemical) certain neurotropic factors are released into the haemolymph. Taurine is the one known to be released into the haemolymph after the vigorous physical activity (Van der Horst et al. 1980, Jabbar & Strong 1985). Perhaps the unidentified compound detected in the present study could be taurine which was released in considerable amount (6-fold in case of malathion) from muscles into the circulating fluid as a result of insecticidal poisoning. What possible role it has in the poisoning of the insect is difficult to visualise with the available data.

A critical scrutiny of the data reveal (table 1) that malathion treatment affected most of the amino acids, the maximum effect being on unidentified compound; followed by pp'-DDT and heptachlor and the least by carbaryl treatment.

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