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Effect of malathion and acetylcholine on the developing larvae of *Philosamia ricini* (Lepidoptera: Saturniidae)

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Abstract. Feeding of malathion induces accumulation of acetylcholine in *Philosamia ricini* developing larvae *via* inhibition of acetyl cholinesterase activity. The insecticide also causes depletion of all nutrients, loss in weight, under-development of silk glands resulting in reduced silk production, lack of oviposition and high mortality among the insects. Acetylcholine however, while fed during the same period of development, tones up their nutritional status, induces better growth, weight gain, improved silk production and oviposition and significantly lower mortality than in the control group of insects maintained on castor leaves. This improved status of insects has been attributed to choline, the insect vitamin released from acetylcholine. Acetylcholine has also been noted to protect the insects to some extent from the poisonous effect of malathion on exposure to it after a dose of acetylcholine during the preceding instar stadium.

Keywords. *Philosamia ricini;* acetylcholine; acetyl cholinesterase; malathion; growth promoter; insecticidal toxicity.

Introduction

Organophosphorus insecticides exert their basic toxicity on the central nervous system *via* inhibition of acetyl cholinesterase by phosphorylating the hydroxyl group of serine in the active site of the enzyme.

Chadwick and Hill (1947) and Smallman and Fischer (1958) confirmed the toxic effect of some lipophilic inhibitors of acetyl cholinesterase in insects and observed a relevant correlation between the sign of poisoning and acetyl cholinesterase inhibition *in vivo.* Thus on the one hand, accumulation of acetylcholine has been shown to poison and induce toxic effects in insects while on the contrary, our observations (Pant *et al.,* 1982) indicate it to be a growth promoter and to some extent as a protector against toxic effects in the lepidopteran phytophagous eri silkworm *Philosamia ricini* when exposed to the chlorofungicide hexachlorobenzene. Therefore, it was considered worthwhile to study the metabolic toxicity exerted by the sulphur-containing organophosphorus insecticide malathion on intermediary metabolism of *P. ricini,* as well as that of acetylcholine-fed insects.

Materials and methods

P. ricini larvae were reared in the laboratory as described earlier (Pant and Agrawal, 1965). At fourth instar stage, larvae were divided into four groups of 500

insects each and lodged in all-round wire-netted wooden cages. Temperature was maintained at 27±2°C and humidity partially controlled as described by Pant and Lacy (1968). Group A was administered acetylcholine, and group M malathion all through fourth and fifth instar stadia, while group B was fed acetylcholine during fourth instar and exposed to malathion in the fifth. The control Group C was maintained all along on *Ricinus communis* leaves.

Before commencing feeding experiments, malathion tolerance was tested on the newly ecdysed larvae through fourth and fifth instar stadia till they pupated. The fatal dose recorded was 90-100 µg/g insect weight/day while the critical dose was 50-60 µg/g insect weight/day.

Malathion and acetylcholine (60 µg each/g insect weight/day) dissolved separately in ethanol were sprayed evenly on fresh castor leaves and exposed to larvae after complete removal of solvent by blowing cold air over the leaves.

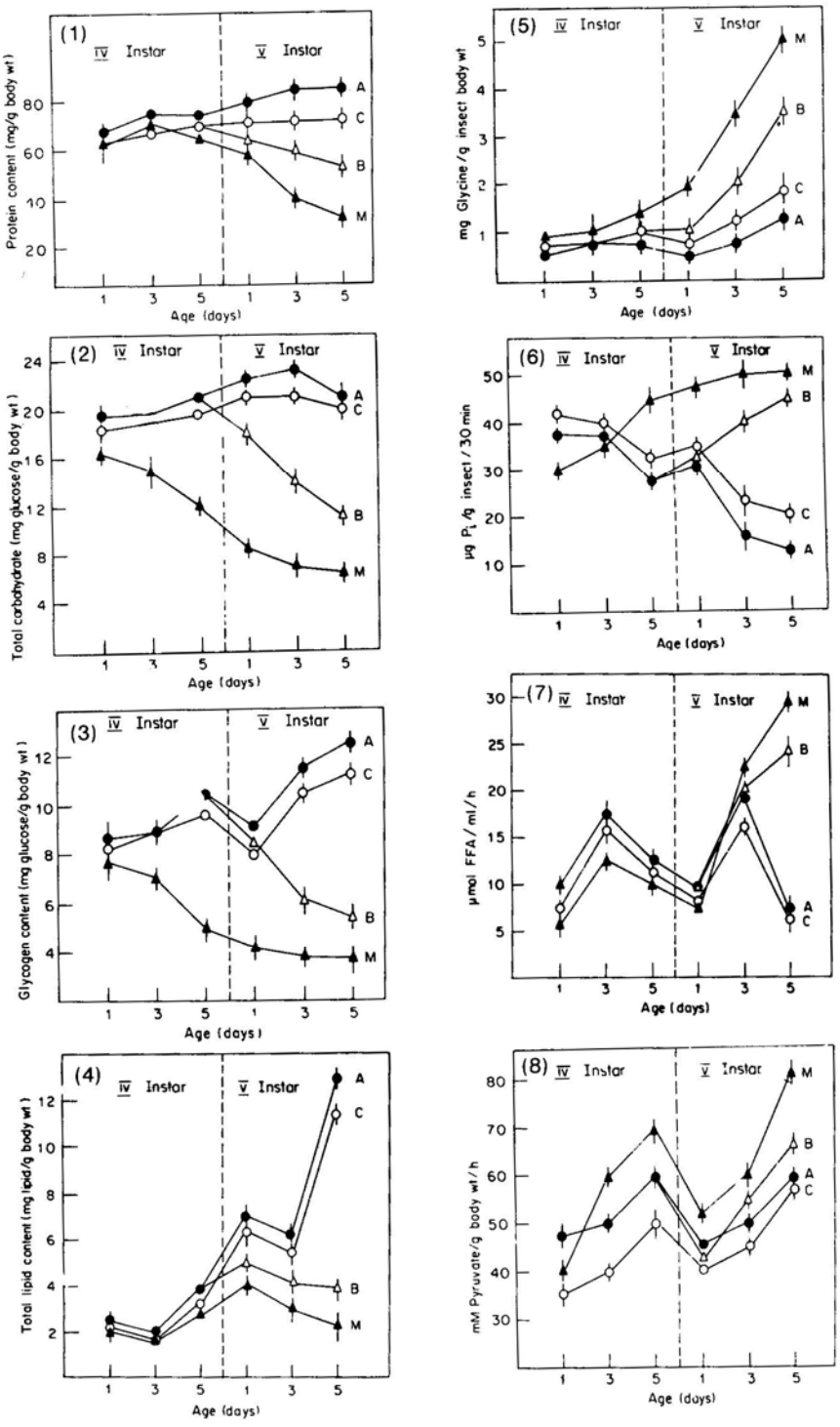
Three lots of 10 larvae each picked randomly from the colony of each group were weighed, chilled in the refrigerator and homogenized to 10% (w/v) tissue concentration in ice-cold glass-distilled water. All assays were carried out in triplicate in each homogenate as well as in the pooled homogenate. The entire experiment was repeated twice over. Although the results did vary quantitatively between 6 and 21%, these variations did not reflect on the variation patterns of the different metabolites and enzymes, observed during development of the insects thus emphasizing the significance of the fluctuations recorded by them.

Proteases were assayed by the method of Matsushita and Iwami (1967) while aspartate (EC 2.6.1.1) and alanine (EC 2.6.1.2) aminotransferases were determined according to Reitman and Frankel (1957). Total free amino acids were estimated by Rosen's method (1957) and proteins by that of Lowry *et al.* (1951). Phosphorylase activity was measured by Green and Stumpfs method (1942), as modified by Srivastava and Krishnan (1961). Lipase activity was assayed by the titrimetric procedure of Roe and Byler (1963). Methods of Ellman *et al.* (1961) was employed to determine the acetyl Cholinesterase activity while for the estimation of acetylcholine the method of Hestrin (1949) as described by Metcalf (1951) was adopted. Total lipids were extracted by the methods of Folch *et al.* (1957) and estimated gravimetrically. Total carbohydrates were determined by the method of Trevelyan and Harrison (1952). Glycogen was isolated as described by Wiens and Gilbert (1967) and estimated by the method of Carrol *et al.* (1956).

Each point in figures represents the mean of three observations in triplicate with upper and lower limits. Insignificant variations have not been shown in figures.

Results and discussion

Insects of A group fed acetylcholine all through fourth and fifth instar stadia recorded higher concentrations of proteins, carbohydrates, glycogen and lipids (figures 1A-4A) than those of the control Group C (figure 1C-4C). This was accompanied with lower activity of proteases, Phosphorylase and lipases (figures 5A-7A). Aminotransferases (figures 8A, 9A) registered lower activity and



Figures 1-8. (for captions see page no. 92).

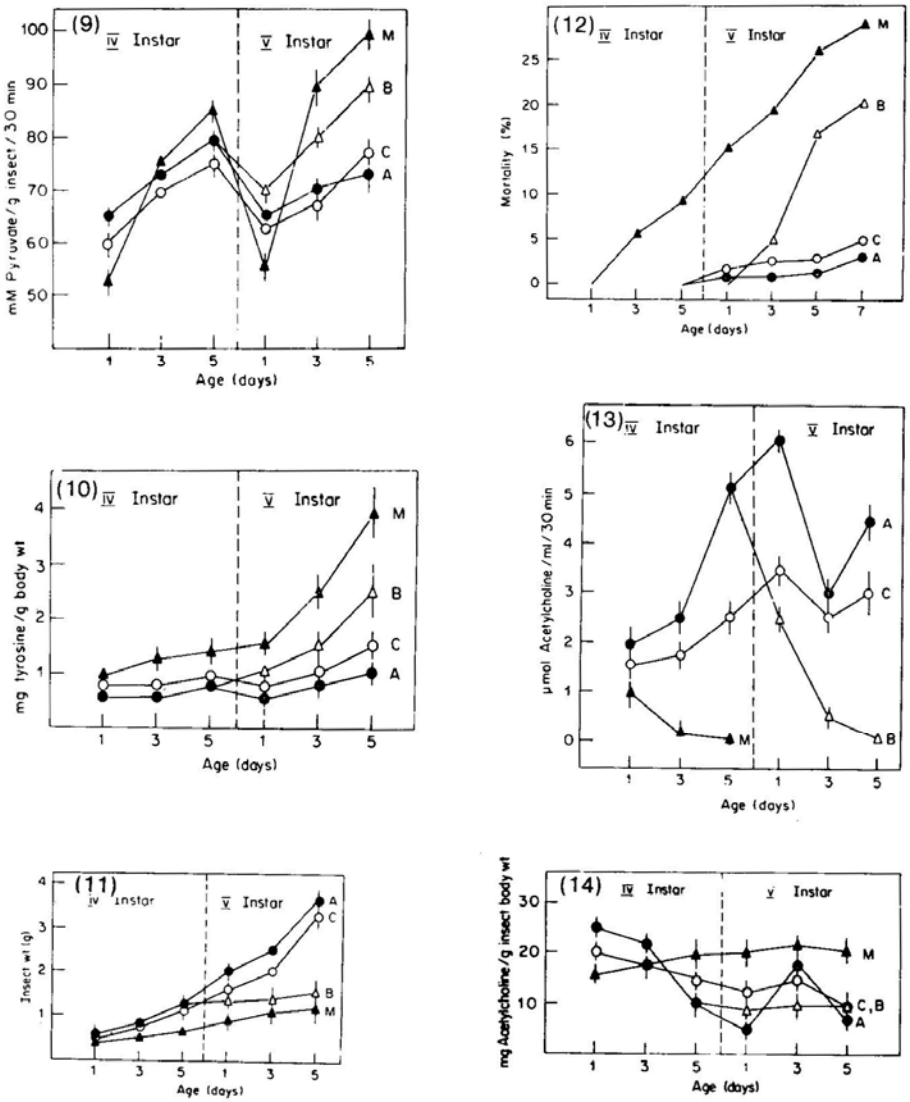


Figure 1-14. The changes in protein **(1);** carbohydrate **(2);** glycogen **(3);** lipids **(4);** protease **(5);** Phosphorylase **(6);** lipase **(7);** aminotransferases **(8** and **9);** free amino acids **(10);** weight loss **(11);** mortality **(12);** acetylcholine esterase **(13)** and acetylcholine concentration **(14)** in acetylcholine and malathion fed *P. ricini.*

accumulated diminished quantities of free amino acids (figure 10A). Feeding of malathion on the other hand, during the same period of development of *P.ricini* larvae, induced opposite and adverse effects. All the afore-mentioned nutrients got depleted significantly (figures IM–4M) with simultaneous enhanced activity of the corresponding enzymes (figures 5M–9M) and increased release of free amino acids (figure 1 0M). In addition, as recorded in table 1, malathion induced

Table 1. Observations made on the fifth instar developing *P. ricini* larva exposed to various conditions of feeding.

Insect feed	Control (C)	Acetylcholine (A)	Malathion (M)	Acetylcholine followed by malathion (B)
Cocoon wt. (gm)	2.06±0.05	2.24±0.06	1.53±0.10	1.76±0.075
% Cocoon formation	93	94	62	78
Wet weight silk gland (mg)	825±5	841±8	207±6	310±10
Length silk gland (cm)	40±2	40±2.5	25±2	30±3
Length of Larva prior to spinning (cm)	6.0±0.6	6.5±0.4	4.2±0.7	5.0±0.5
Wt. of larva prior to spinning (gm)	3.25±0.15	3.7±0.20	1.2±0.20	1.6±0.10

weight loss (also represented in figure 11M) and stunted growth, under-development of silk glands with impoverished capacity to spin silk and a very high rate of mortality (figure 12M). No moth emergence occurred and those that emerged did not oviposit.

On the other hand, the insects of group B fed acetylcholine earlier and then exposed to the action of malathion appeared to thrive better in every respect (figures 1B– 14B) than the insecticide-fed M group of insects. Mortality rate was also significantly lower (figure 12B). These observations indicate that acetylcholine is protecting the insects to certain extent from the poisonous action of the insecticide. This could be correlated to the acetyl cholinesterase activity (figure 13A) in the acetylcholine-fed group A and acetylcholine concentration (figure 14A) where high activity of the enzyme and low concentration of acetylcholine have been noted. It is assumed that the choline released by acetyl cholinesterase improves the nutritional status of the insects, promotes better growth (figure 11 A) and also combats to some extent against malathion poisoning.

Acetylcholine concentrates maximally in group M (figure 14M) than in any

other group. The low acetyl cholinesterase activity further declines gradually with development and at fifth instar stage becomes totally inactive (figure 13M) leading to the high accumulation of acetylcholine (22.3 mg/g insect weight, figure 14M). This perhaps results in the loss of muscular co-ordination followed by convulsions and ultimate death of the insects.

The prolonged fourth instar stage by 2-3 days in the insecticide-fed group M suggests disturbance of hormonal equilibrium. Malathion not only destroys insects but also leaves in them a long-standing effect if survived resulting in their destruction by affecting the reproductive function viz., oviposition.

For proper growth and development, choline has been shown as an essential constituent in the larval diet of several insects belonging to several orders and a few species of lepidoptera like the *Bombyx mori* (Horie and Ito, 1965), *Agrotis orthogonia zea* (Kastings and McGinnis, 1967), *Heliothes zea* (Vanderzant, 1968) and *Angyrotaenia velutinana* (Rock 1969) etc. Although *P. ricini* has not yet been experimented upon for its absolute dependence on choline for normal growth, the present investigation provides ample evidence to the fact, that feeding of acetyl choline improves growth, development, oviposition and silk production etc., under normal conditions and during stress, functions as a protector from adverse effects.

The fact that on the one hand acetyl choline proves beneficial to insects and on the other, accumulation thereof in them produces toxicity is rather intriguing. While its accumulation could be due to the inhibition of acetyl cholinesterase activity by malathion, its role as a protector to insects against insecticidal toxicity could be traced to choline released from acetyl choline which tones up the nutritional status of insects.

This speculation is well supported by several other reports (Hayes, 1975; Gains, 1960; Webb *et al.,* 1973) on malathion toxicity that factors like temperature, environment, dosage and mode of exposure of animals as well as the species and their nutritional status modify the toxicity.

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