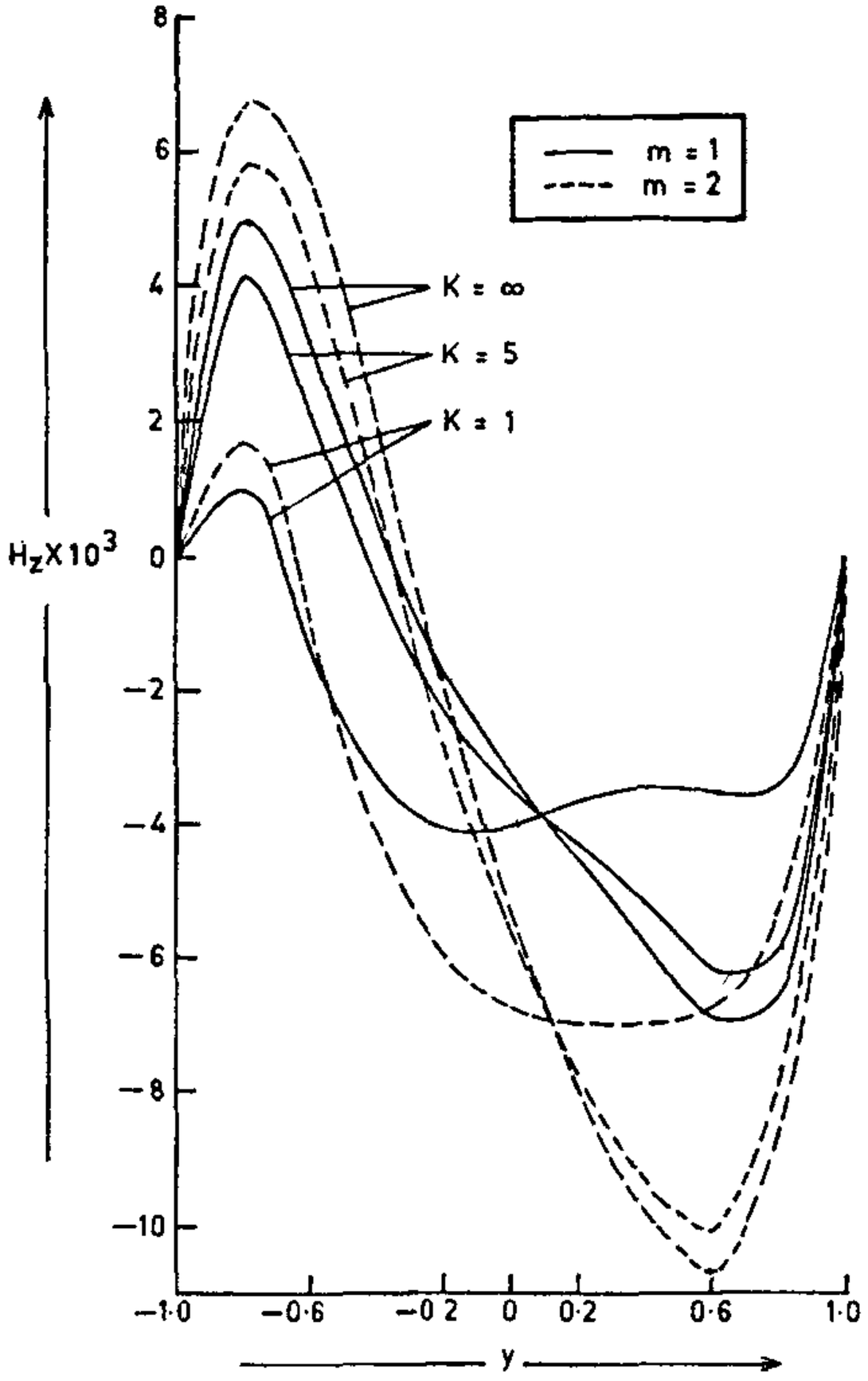
FIG. 4 PROFILES OF NON-DIMENSIONAL INDUCED MAGNETIC FIELD H_z FOR M = 5 AND G = 1



for the primary and cross-flow increase numerically with the increase in Hall parameter (m). Hence there is no possibility of the flow separation at both the plates when $G = 1$, $K = 1$ and $M = 5$.

TABLE 1

Values of shear stress

m	$\left(\frac{du}{dy}\right)_{y=1}$	$\left(\frac{du}{dy}\right)_{y=-1}$	$\left(\frac{dw}{dy}\right)_{y=1}$	$\left(\frac{dw}{dy}\right)_{y=-1}$
1.0	−0.740408	1.094737	0.101272	−0.197663
2.0	−0.778453	1.197591	0.153687	−0.293120
3.0	−0.800944	1.275771	0.189259	−0.338404

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EFFECT OF FEEDING HEXACHLOROBENZENE AND ACETYLCHOLINE TO *PHILOSAMIA RICINI* LARVAE DURING DEVELOPMENT

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ABSTRACT

The fungicide, hexachlorobenzene, acts as a nerve poison to *Philosamia ricini* larvae by inhibiting acetylcholine esterase activity and producing toxicity. These lead to the lysis of all nutrients—carbohydrates, glycogen, proteins and lipids as evinced by the enhanced proteolytic, lipolytic, phosphorylase and aminotranferases activities throughout the development of *P. ricini*. It also induce lack of appetite and renders the insects undernourished. Release of total free amino acids, due to the high proteolytic activity could also account for the high mortality rate (47%) of the fungicide-fed insects due to amino acidaemia. Feeding of hexachlorobenzene to acetylcholine fed insects produced more or less the same overall effect as with the fungicide alone.

INTRODUCTION

EARLIER studies on the effect of insecticides on plant tissues[1-3] and yeast cells[4] induced the authors to explore the action of a fungicide on insects. Therefore, in the present investigation *P. ricini*, the silk producing Eri worm larvae were subjected to the action of hexachlorobenzene—a fungicide employed for protecting seeds of plants from fungal infection. Since chlorinated compounds are known to induce acetylcholine depletion and affect the turnover of catecholamines in insects[5,6], both normal as well as acetylcholine-fed larvae were exposed to the fungicidal action.

MATERIALS AND METHODS

Viable eggs of *P. ricini* procured from Ericulture Institute, Nathnagar, Bhagalpur, were spread on filter paper in clean petri dishes. The newly emerged larvae were reared on fresh tender castor leaves. At the fourth instar stage, the larvae were divided into four groups of 500 insects each, and lodged in wooden all round wire-netted cages in trays. Temperature was maintained at 27 ± 2°C and humidity partially controlled by placing water-soaked cotton pads in enamel bowl in the cage. Group A was administered acetylcholine, group B hexachlorobenzene and group C acetylcholine during fourth instar development followed by hexachlorobenzene in the fifth instar. A control group D was also maintained simultaneously only on fresh *Ricinus communis* leaves.

The fungicide and acetylcholine (75 μg each/g fresh body weight of larvae) dissolved respectively in benzene and ethanol, were evenly sprayed on castor leaves and exposed to the larvae after complete removal of the organic solvent by blowing cold air on the leaves.

Three lots of 8 randomly picked larvae from each group were weighed, washed with ice-cold distilled water and refrigerated for 60 minutes.

Three lots of 10 washed and well-dried chilled larvae, selected at random from each group were quickly decapitated, the severed head parts collected separately in chilled beakers, quickly weighed and refrigerated for 60 minutes. Both the entire larvae and the head homogenates were prepared in chilled glass-distilled water to 10% (w/v) tissue concentration. All assays were made in triplicate of each homogenate as well as of the pooled homogenates.

Aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) activities were assayed by Reitman and Frankel's[7] method while proteolytic activity by that of Matsushita and Iwami[8]. Phosphorylase activity was determined by Green and Stumpf's[9] method as modified by Srivastava and Krishnan[10]. Lipase activity was assayed by the titrimetric procedure of Roe and Byler[11]. For determining acetylcholine esterase activity, Ellman's method[12] was employed while acetylcholine was estimated according to Hestrin's method[13] as described by Metcalf[14]. Protein was estimated according to the method of Lowry[15] while total free amino acids were determined by Rosen's method[16]. Glycogen was isolated by Wien and Gilbert's method[17] and estimated by that of Carrol's[18]. Total carbohydrates were determined by Trevelyan and Harrison's method[19].

Total lipids were extracted with chloroform-methanol mixture (2:1, v/v) employing 10 ml of the solvent mixture per ml homogenate. The extraction was repeated twice and the pooled extract was treated with 1 volume of NaCl (0.79%, w/v) in a separating funnel. The lower phase containing the lipids was concentrated *in vacuo* and dried to constant weight. Results are represented in figures 1–6 as well as in table 1. Each point in the figures represents the mean of three observations in triplicate with upper and lower limits. Insignificant variation limits have not been shown in the figures.

RESULTS AND DISCUSSION

Administration of acetylcholine to *Philosamia ricini* larvae appears to induce enhanced protein biosynthesis (figure 1a), low proteolytic activity (figure 2a) and low total free amino acids concentration (figure 3a). Aminotransferases activity (table 1), total carbohydrates (figure 4a) and glycogen content (figure 5a) stood at a higher level than in the control group (figures 4d–5d) of insects all through the fourth and fifth instar development till the commencement of spinning.

Acetylcholine esterase activity increased markedly in groups A and D (table 1) during the fourth instar entire larvae till ecdysis to the fifth instar and declined significantly on day 3 in all groups. Enhanced activity of the enzyme in group A head part of *P. ricini*, in comparison to those of groups B and C was noted only on day 3 during both fourth and fifth instar stages while acetylcholine concentration did not appear to be appreciably at variance from the other groups both in the entire larvae and the head parts thereof. Results are shown in table 1.

Feeding of hexachlorobenzene resulted in gradual protein depletion (figure 1b), with simultaneous enhanced proteolytic activity (figure 2b), high aminotransferases activity and in significant increase in total free amino acids (figure 3b). Both total carbohydrates and glycogen content gradually declined accompanied with enhanced phosphorylase activity especially on days 3 and 5 of the fifth instar larva (figures 4b, 5b and 6b). Acetylcholine content of the entire body tissue and of the head part of group B insects did not appear to have been influenced by feeding of hexachlorobenzene as observed from their more or less constant concentration.

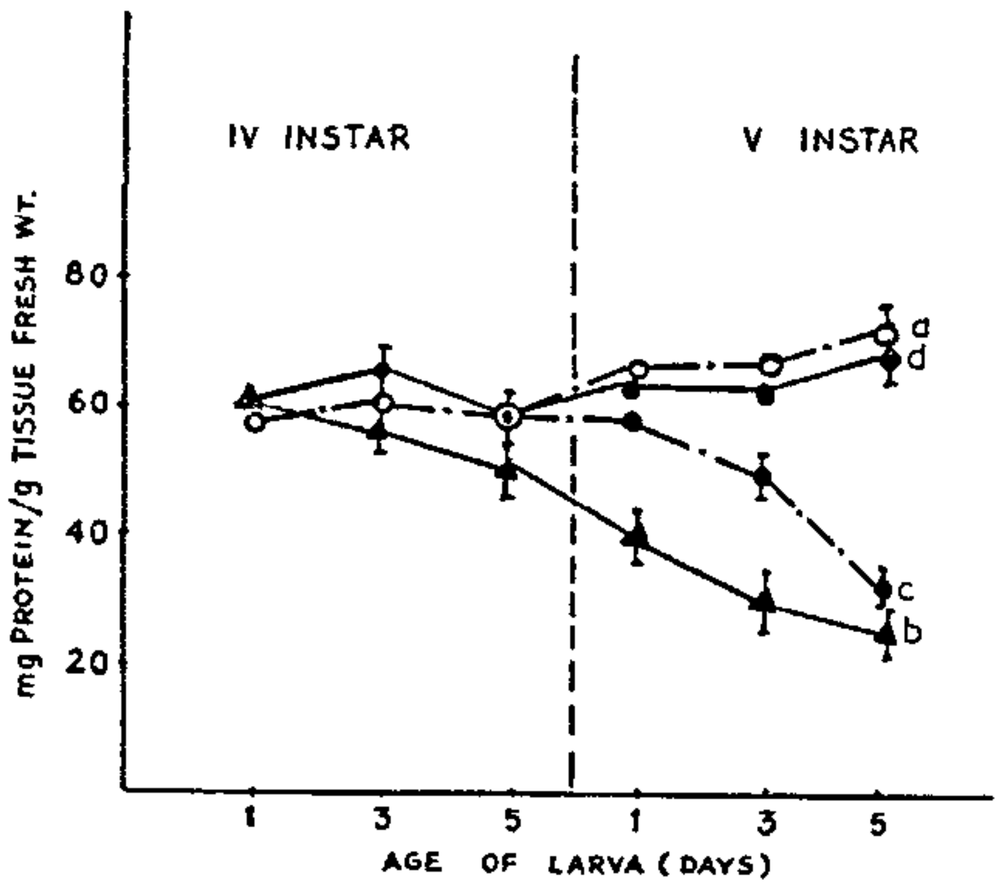

FIG. 1. VARIATION IN PROTEIN CONTENT

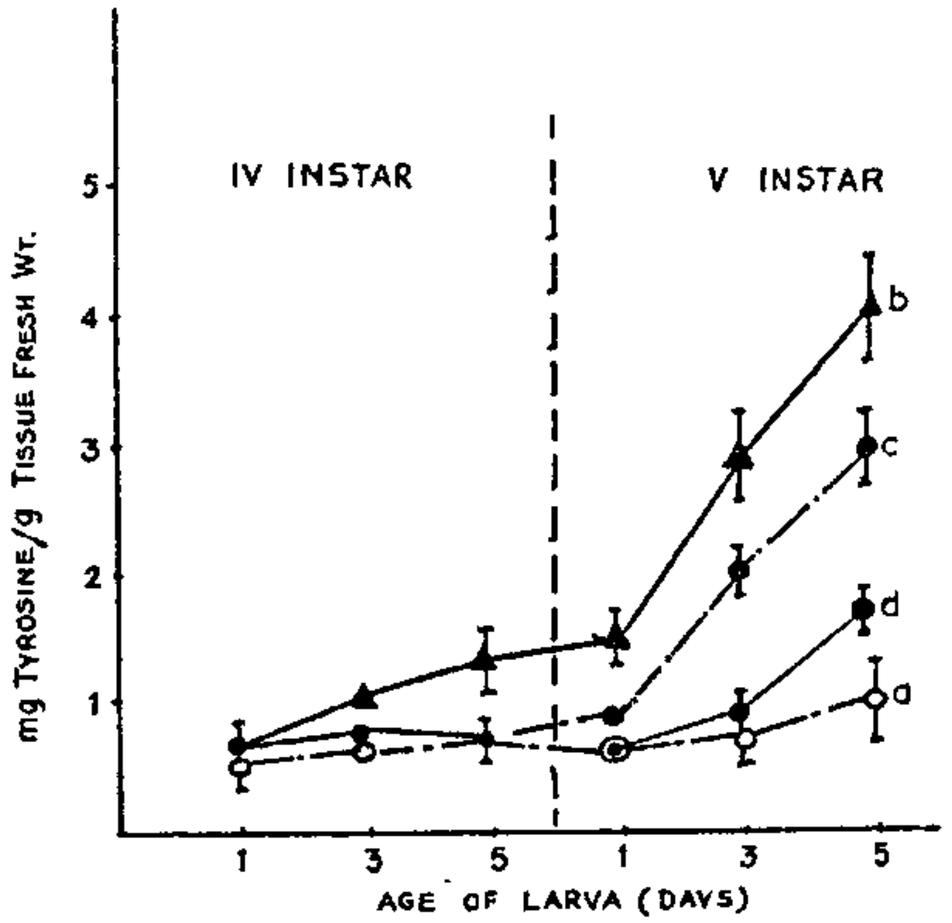

FIG. 2. VARIATION IN PROTEOLYTIC ACTIVITY

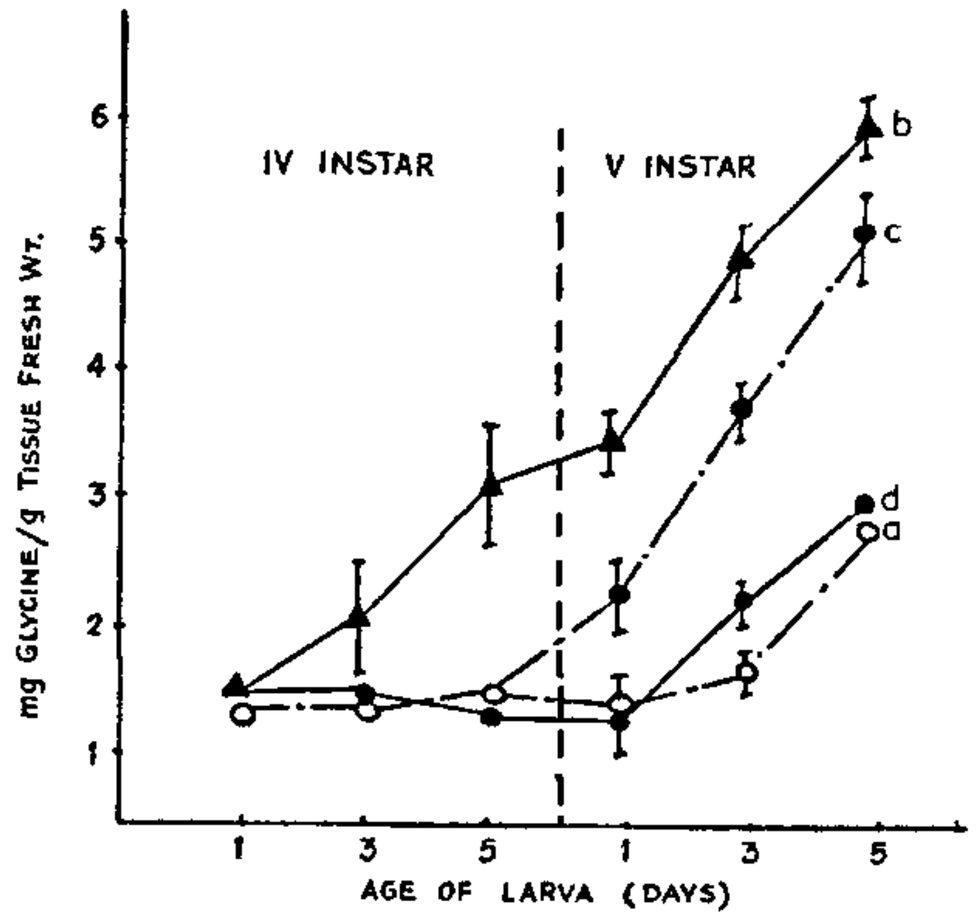

FIG. 3. VARIATION IN TOTAL FREE AMINO ACIDS

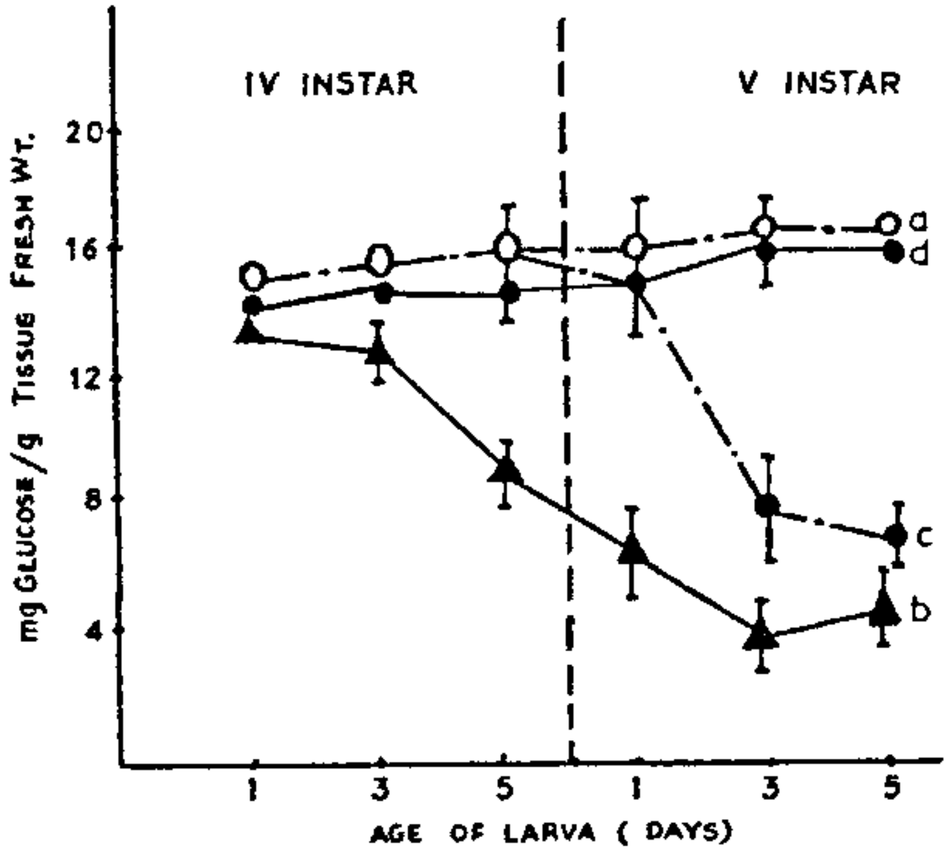

FIG. 4. VARIATION IN TOTAL CARBOHYDRATES

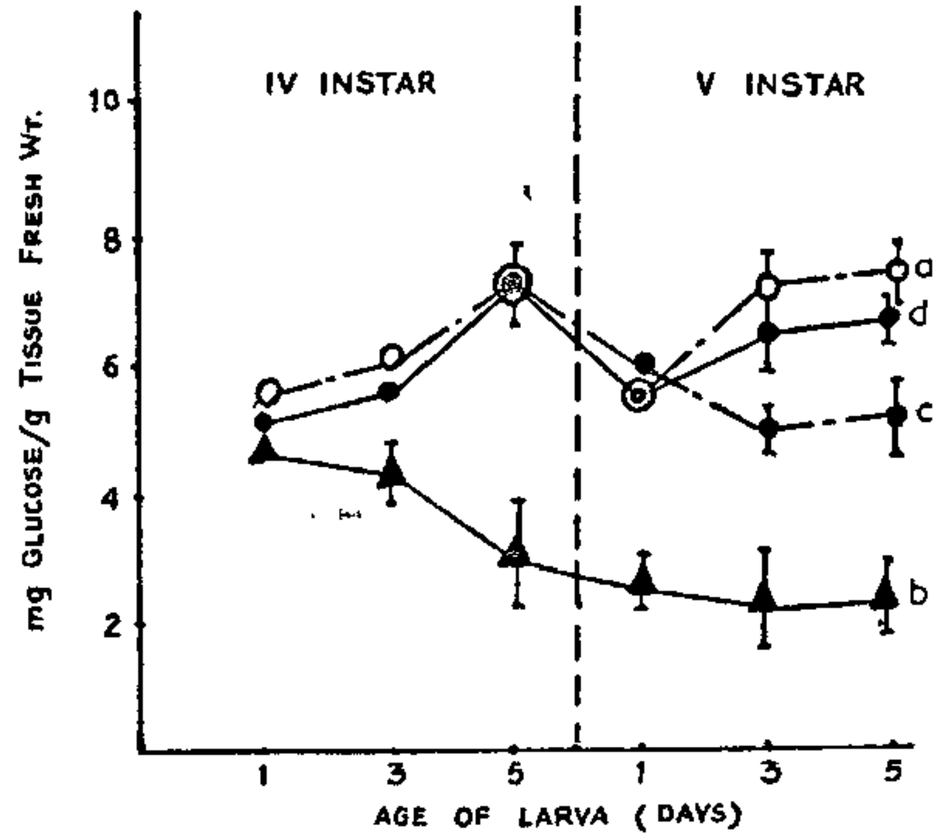

FIG. 5. VARIATION IN GLYCOGEN

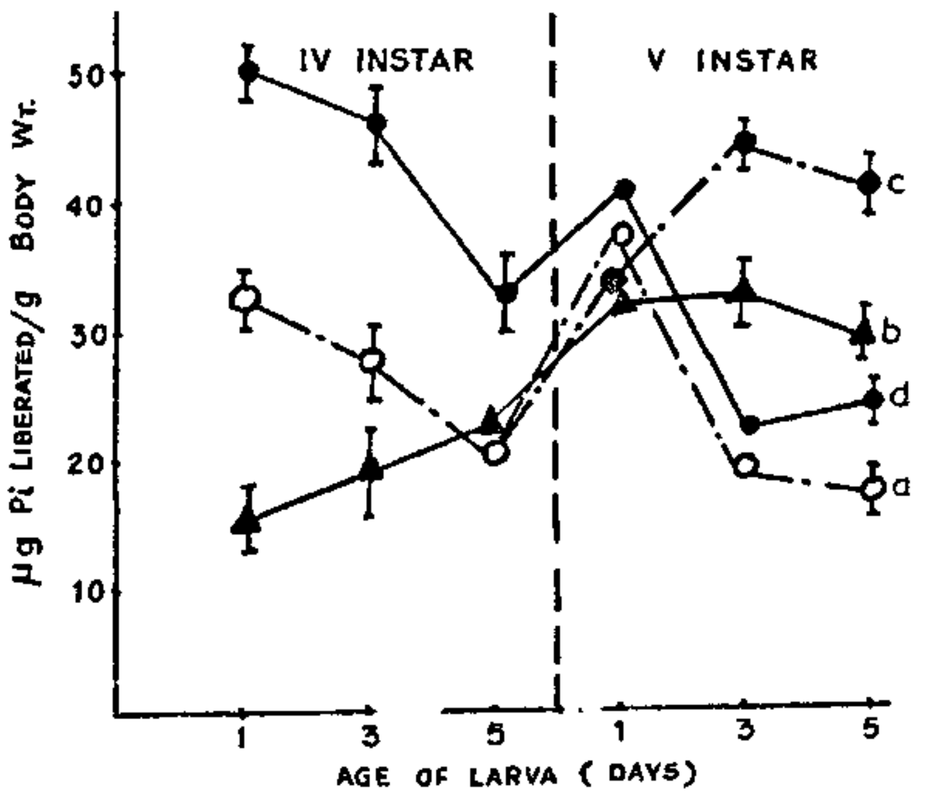

FIG. 6. VARIATION IN PHOSPHORYLASE ACTIVITY

a = ACETYLCHOLINE-FED GROUP
b = HEXACHLOROBENZENE-FED GROUP
c = ACETYLCHOLINE + HEXACHLOROBENZENE-FED GROUP
d = CONTROL GROUP (*Ricinus communis* LEAVES-FED GROUP)

Feeding of the fungicide hexachlorobenzene enhanced lipase activity (1.0-36.0 μ moles FFA) in *P. ricini* as borne out by the simultaneous low lipid level (6.9-1.7 mg/g tissue). This assumption is lent support by the lipase and lipid profiles in group C of the insects where the net lipase activity stands higher than in insects of groups A and D. Further, feeding of the fungicide alone and when combined with acetylcholine, induced high insect mortality rates of 47% and 40% respectively. In addition, the fungicide adversely affected the appetite of the larvae as noted from their aversion to the feed—*Ricinus communis* leaves. This lead to starvation and stunted growth and development in insects which ultimately resulted in their total loss of cocoon spinning capacity.

On the other hand, group A exposed to acetylcho-

TABLE 1

Effect of feeding hexachlorobenzene and acetylcholine to Philosamia ricini larvae (Dosage: Hexachlorobenzene and Acetylcholine 75 µg each/g body weight)

		4th instar			5th instar		
Days		1	3	5	1	3	5
GOT (mM pyruvate/g body weight)	D	35 ±4	43 ±2	55 ±2	45 ±2	42 ±2	55 ±3
	B	36 ±3	55 ±2	80 ±2	60 ±5	72 ±5	80 ±4
	A	40 ±5	47 ±3	60 ±4	55 ±3	57 ±4	60 ±3
	C	50 ±5	47 ±3	60 ±4	57 ±2	63 ±5	70 ±5
GPT (mM pyruvate/g body weight)	D	68 ±3	72 ±2	74 ±4	60 ±3	62 ±3	64 ±5
	B	55 ±2	78 ±4	90 ±3	85 ±3	90 ±4	100 ±6
	A	72 ±4	80 ±2	81 ±5	75 ±4	78 ±5	80 ±2
	C	72 ±4	80 ±2	81 ±5	72 ±6	78 ±4	85 ±3
Lipase activity (μ moles/ml/hr)	D	6.6 ±1	17.5 ±1	28.2 ±2	6.3 ±0.5	12.5 ±1	16.7 ±2
	B	1.0 ±0	1.4 ±0.5	20.0 ±2	24.2 ±1	27.0 ±2	36.0 ±4
	A	12.2 ±1	15.0 ±2	27.0 ±3	18.3 ±1	25.3 ±3	20.0 ±3
	C	12.2 ±1	15.0 ±2	27.2 ±3	17.0 ±2	24.0 ±2	30.0 ±5
Total lipids (mg/g fresh weight)	D	12.3 ±3	6.5 ±1	2.3 ± 0.5	2.0 ±0.02	4.3 ± 0.1	4.4 ±1
	B	0.2 ±0	6.9 ±1.5	2.2 ± 0.4	1.7 ±0.1	3.9 ± 0.5	1.7 ±0.6
	A	7.0 ±1	6.3 ±2	2.2 ± 0.5	1.0 ±0.03	4.6 ± 1	5.0 ±1
	C	7.0 ±1	6.3 ±2	2.2 ± 0.5	2.7 ± 0.2	2.7 ± 0.5	1.0 ±0.4
Acetylcholine esterase (μ mol ACh/ml/ 30 min)	D	1.65 ± 0.3	0.2 ±0	0.6 ±0.1	4.0 ±0.2	0.1 ±0	2.1 ± 0.1
	B	1.68 ± 0.3	0.7 ± 0	0.1 ± 0	0.0 ± 0	0.2 ± 0.0	1.4 ± 0.1
	A	1.85 ± 0.4	0.3 ± 0.1	2.2 ± 0.05	6.6 ± 0.2	1.2 ± 0.01	1.3 ± 0.05
	C	1.85 ± 0.4	0.3 ± 0.1	2.2 ± 0.05	0.0	0.0	4.1 ± 0.3
Acetylcholine (mg ACh/g fresh weight)	D	23.0 ± 4	17.1 ± 2	20.0 ± 2	21.2 ± 2	22.0 ± 1	16.6 ± 2
	B	14.6 ± 3	20.8 ± 3	24.2 ± 4	25.9 ± 1	21.8 ± 1	23.1 ± 3
	A	22.5 ± 2	21.8 ± 3	24.3 ± 1	21.1 ± 2	30.1 ± 2	32.3 ± 4
	C	22.5 ± 2	21.8 ± 3	24.3 ± 1	17.1 ± 3	26.1 ± 2	29.6 ± 2
Acetylcholine esterase (Head) (μ mol ACh/ml 30 min)	D	3.75 ± 0.1	0.39	1.6 ± 0.06	0.0	0.0	0.0
	B	0.0	0.81	0.0	0.0	0.0	0.0
	A	2.09 ± 0.05	2.75 ± 0.1	0.88	0.0	0.55	0.0
	C	2.09 ± 0.1	2.75 ± 0.2	0.88	0.0	0.0	0.0
Acetylcholine (Head) (mg ACh/g fresh weight)	D	54.5 ± 5	31.8 ± 5	30.8 ± 3	16.3 ± 2	46.1 ± 5	39.0 ± 2
	B	42.8 ± 6	43.4 ± 3	32.7 ± 2	13.7 ± 2	37.1 ± 4	30.1 ± 4
	A	36.3 ± 4	33.0 ± 4	37.3 ± 4	17.8 ± 3	34.2 ± 2	32.7 ± 3
	C	36.3 ± 4	33.0 ± 4	37.3 ± 4	7.8 ± 1	31.2 ± 3	30.2 ± 2

Table 1—(*Continued*)

		4th instar			5th instar			
Days		1	3	5	1	3	5	7
Mortality Rate (%)	D	nil	nil	nil	2.6	6.0	14.0	14.0
	B	nil	1.6	3.5	8.6	18.0	29.0	47.6
	A	nil	nil	nil	nil	2.5	5.0	11.5
	C	nil	nil	nil	nil	5.0	11.0	40.0
Weight change (g/insect)	D	0.6	0.72	1.36	2.6	2.8	3.0	
	B	0.6	0.68	0.76	1.07	1.70	0.9	
	A	0.6	0.72	1.65	2.2	2.7	3.05	
	C	0.6	0.72	1.65	1.2	1.4	1.55	

A = Acetylcholine-fed group; B = Hexachlorobenzene-fed group; C = Acetylcholine + Hexachlorobenzene-fed group; D = Control group (*Ricinus communis* leaves-fed group); ACh = Acetylcholine.

line alone, developed normally into healthy larvae and spun silk at par with the control group D.

Thus it can be concluded that the fungicide hexachlorobenzene has an insecticide like nerve poisoning action on *P. ricini* larvae by inhibiting acetylcholine esterase activity. In all probability, the inhibited esterases induced toxicity in them reflecting their lack of appetite and undernourishment. These further lead to the lysis of all nutrients—carbohydrates, glycogen, proteins and lipids as evinced by the enhanced proteolytic, lipolytic, phosphorylase and aminotransferases activities.

Choline is known to be a vitamin in insects which enhances their growth and development. Feeding of acetylcholine rapidly increases acetylcholine esterase activity resulting in the release of choline which perhaps promotes growth and development. Since feeding of additional supplies of acetylcholine to insects did not alter its concentration significantly in them but only resulted in enhanced acetylcholine esterase activity, the speculation that acetylcholine gets catabolized to choline receives further support. This perhaps also explains the enhanced protein synthesis and increased concentrations of carbohydrates and glycogen in the acetylcholine-fed group of insects.

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