

embedded between the degenerating cells. The number of Leydig cells seems to be normal; however, they are compressed between the tubules. Some of the central tubules lose their lumen and show only a few closely packed spermatids.

With the administration of 1 ml of 0.8 mM solution of uranyl acetate the general structure of the testes shows shrinkage so that the tubules show compact arrangement (figure 3). The lumen of most of the central tubules is reduced, while most of peripheral tubules have spermatozoa. Under this treatment the number of spermatids was considerably reduced and in some tubules no spermatid was found (figure 3). The number of Leydig cells does not seem to be affected.

The administration of 1 ml of 0.4 mM solution of uranyl acetate could not induce significant changes in the testicular region.

Figure 4 shows the normal histological findings in the uterus of adult bat. With the administration of 1 ml of 1 mM solution of uranyl acetate to female bats, the uterine gland is enlarged but no secretion is found in the lumen (figure 5). The cells of the glands have lost their eosinophilic nature and in some glands atrophic changes are observed with hyalinization of the epithelium. Uterine vascularity has increased considerably.

With the administration of 1 ml of 0.8 mM solution of uranyl acetate, the lumen of the uterine glands is small (figure 6). The epithelium has elongated nuclei. The glands show normal secretion. Some cells undergo liquifaction. The nuclei show some degree of hypertrophy.

The administration of 1 ml of 0.4 mM solution of uranyl acetate did not induce significant change in the uterine glands.

The present investigation thus reveals that the administration of uranyl acetate to adult male bat caused severe damage to the testis as revealed by histological studies. These changes include inflammation of tunica albuginea, disorganization of germ cells and their exfoliation. The blood vessels also show dilation. Our observation support the findings of Kamboj and Kar<sup>3</sup> who have reported that salts of uranium caused damage and lysis of the cellular element along with disorganized interstitium, vascular enlargement and destruction of Leydig cells. General shrinkage of the testis and deformity of the seminiferous tubules suggest that the administration of uranyl acetate has some effect on the fluid transport mechanism of the testis as it has already been suggested that metals may cause disturbances in fluid movement<sup>4</sup>. Furthermore, the fibrosis, necrosis, chromolysis and

other severe damages observed in the present study may be caused by the disturbances in the cell physiology and its metabolism as the metal ions may form complexes with key ligands thereby poisoning the cellular enzyme system<sup>4,5</sup>.

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#### EFFECT OF DIFFERENT DOSAGES OF ACETYLCHOLINE ON GROWTH, OVIPOSITION AND SILK PRODUCTION OF *BOMBYX MORI* [LEPIDOPTERA: SATURNIIDAE]

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REPRODUCTION and oviposition of insects are considered to be influenced by different proteins. It has been shown by several investigators<sup>1-5</sup>, that choline is an essential constituent in the larval diet for proper growth and development. Different chemical compounds like Hempa, Thiotepa, hexachlorobenzene, malathion and hexamethylmelamine, when injected or fed to the insects, induce inhibition of enzyme acetylcholinesterase causing accumulation of acetylcholine<sup>5-7</sup>. Thus on the one hand, choline released from acetylcholine promotes better growth and development, while on the other, (the accumulation of acetylcholine in the tissues causes toxic effects. However, detailed work has not been done to study the effect of higher dosages of acetylcholine in *Bombyx mori*. The effect of different dosages of acetylcholine on the growth and development of larvae of *Bombyx mori* is reported in this note.

Eggs of *Bombyx mori* were spread on filter paper. The newly emerged larvae were reared on fresh tender mulberry leaves at 26°C ( $\pm 2$ ). After the fourth moult, (i.e. fifth instar stage), the larvae were divided into six groups of 150 each, and were kept separately in wooden trays. Group A to E were respectively fed 40, 60, 80, 100 and 120  $\mu\text{g}$  of acetylcholine per gram weight of body. A control group X was also maintained simultaneously on fresh mulberry leaves.

Acetylcholine, dissolved in ethanol, was sprayed on mulberry leaves with an atomizer, then the organic solvent was completely removed by blowing cold air over the leaves after which the leaves were fed to larvae. Acetylcholine-treated leaves were fed once daily early in the morning.

Fifteen larvae picked randomly from each group were weighed, chilled in the refrigerator and homogenized in ice-cold double distilled water. This was done on first, third, fifth and seventh day of the fifth instar larvae. Acetylcholinesterase activity was determined by Ellman method<sup>8</sup> and acetylcholine was estimated by the method of Hestrin<sup>9</sup> as described by Metcalf<sup>10</sup>.

Larvae of groups A, B and C showed marked increase in their body weights (table 1) than the control

group X, while the larvae of group D and E were weaker compared to other groups. The rate of oviposition and silk quantity (dry weight of cocoon) was greater in groups A, B and C than X, but in the case of groups D and E, it decreased (table 2). These observations indicate that choline, known to be an insect vitamin released from acetylcholine by acetylcholinesterase activity, enhances their growth and development.

Several investigators<sup>1-4,7</sup> have shown that choline is an essential constituent for proper growth and development. The above result can be correlated with the acetylcholinesterase activity and acetylcholine concentration in tissues of groups A, B and C where there was gradual decrease in the concentration of acetylcholine (figure 1) and increase in the activity of acetylcholinesterase enzyme than the control group X (figure 2). However, groups D and E showed just the reverse. There was decrease in the growth of larvae, oviposition, rate of hatching, silk production (table 2) and the concentration of acetylcholine in the tissues was high (figure 1).

Earlier studies<sup>5,7,11,12</sup> had shown that different chemicals induce accumulation of acetylcholine in the tissues due to inhibition of acetylcholinesterase ac-

Table 1 Effect of acetylcholine on larval growth

Dose of Acetylcholine/g body wt. ( $\mu\text{g}$ )	Weight of larvae in g of Vth instar						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Control (X)	0.618	0.956	1.096	1.755	2.158	2.169	2.292
40 (A)	0.618	0.986	1.240	1.171	1.802	1.189	2.313
60 (B)	0.618	1.041	1.310	1.866	2.457	2.457	2.623
80 (C)	0.618	1.123	1.482	2.150	2.733	2.733	3.103
100 (D)	0.618	1.119	1.291	1.817	2.171	2.163	2.138
120 (E)	0.618	1.019	1.034	1.059	1.081	1.085	1.097

Table 2 Effect of acetylcholine on oviposition, rate of hatching and silk quantity

Amount of dose/g body wt. ( $\mu\text{g}$ )	Average no. of eggs laid/larvae	Average no. of eggs hatched/larvae	Percentage of hatching	Dry wt. of cocoon in mg.
Control (X)	494	437	88.46	292.0
40 (A)	502	448	89.2	294.6
60 (B)	539	490	91.04	309.9
80 (C)	574	538	93.8	348.5
100 (D)	491	316	64.5	258.0
120 (E)	403	246	61.04	187.5



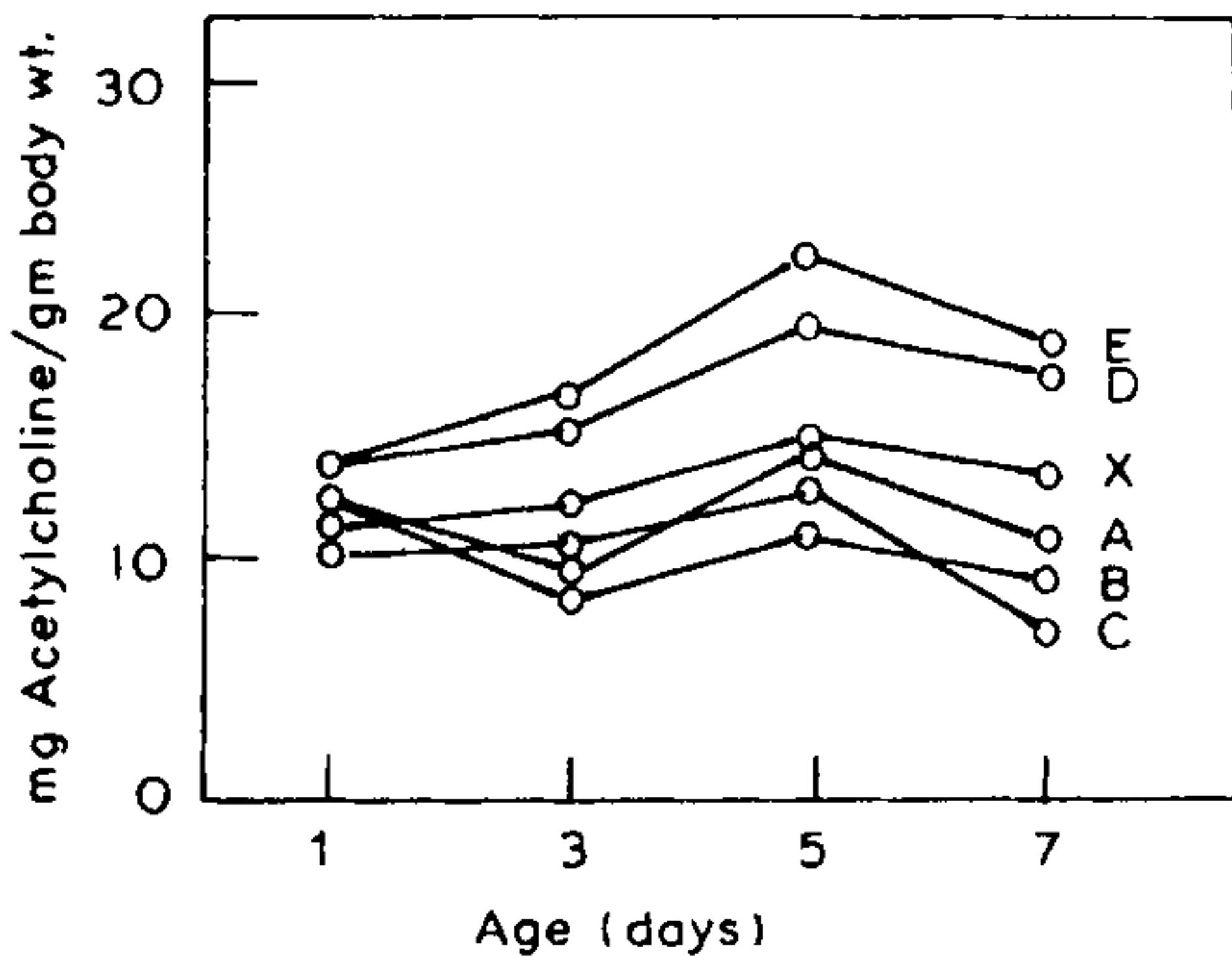


Figure 1. Changes in the acetylcholine concentration in the fifth instar stage.

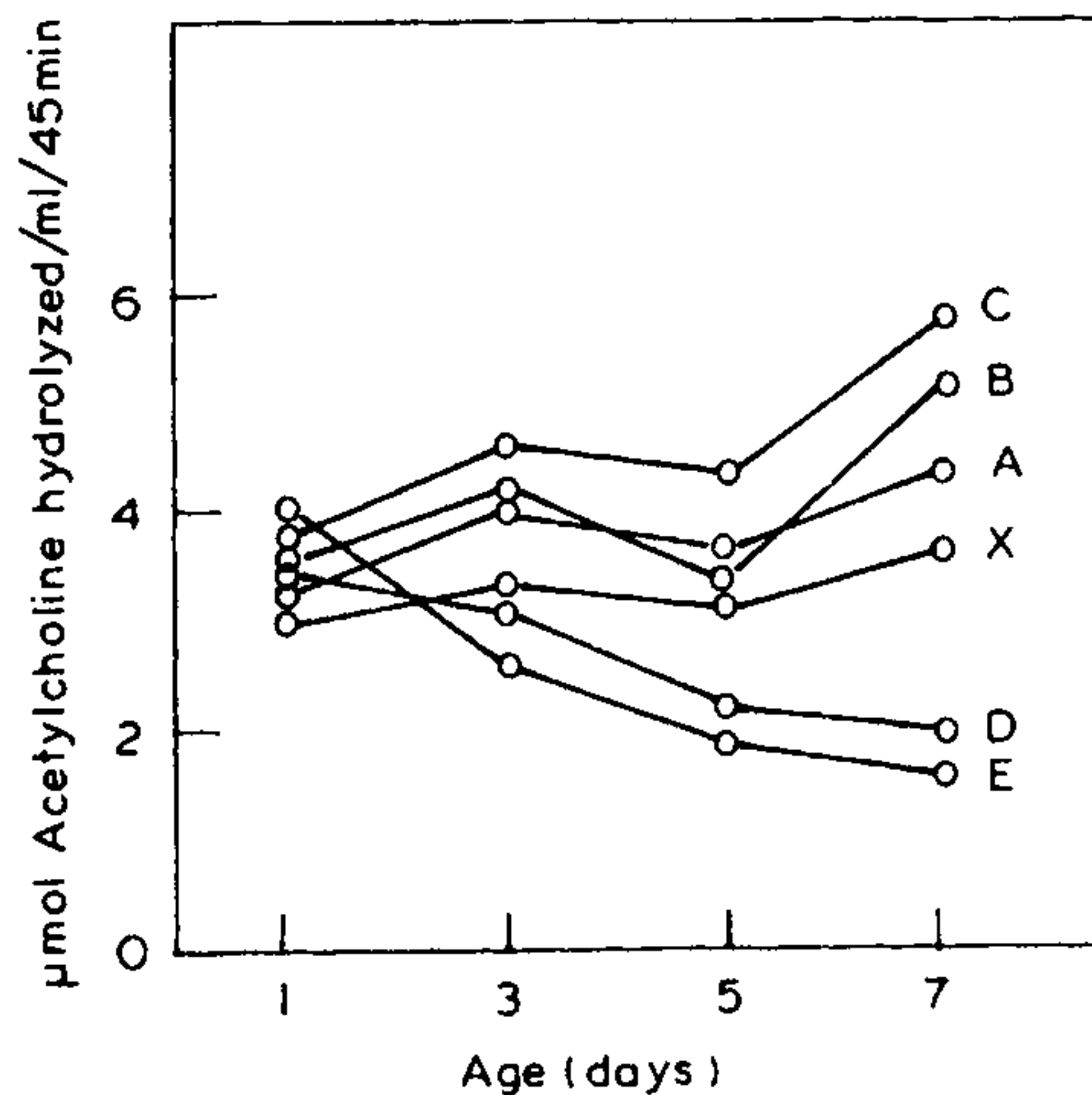


Figure 2. Changes in the acetylcholinesterase activity in the fifth instar stage.

choline due to the inhibition of acetylcholinesterase enzyme by malathion, causes depletion in nutrients, loss in weight, underdevelopment of silk glands resulting in reduced silk production, decrease in oviposition and rate of hatching.

The present study shows that the higher dosages of acetylcholine inhibit the acetylcholinesterase enzyme resulting in accumulation of acetylcholine and causing retarded growth and reduction in oviposition, rate of hatching and silk production. The present investigation provides ample evidence to the fact that feeding of acetylcholine on the one hand improves the growth and development, oviposition and silk production by enhancing the acetylcholinesterase activity and on the other, high dosages inhibit the enzyme causing accumulation of acetylcholine thus producing reverse effects.

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tivity, which becomes toxic in insects. Kuribayashi<sup>13</sup> showed that exposures of *Bombyx mori* larvae to sublethal doses of organophosphorus insecticides such as parathion and disulphoton, induced specific ovicidal action on the development of eggs. The embryos of eggs laid, died just after hatching due to the inhibition of the embryonic cholinesterase activity. Pant and Katiyar<sup>7</sup> showed that the accumulation of acetyl-