EFFECT OF POLYHEDROSIS ON THE HAEMOLYMPH TRANSAMINASES IN THE SILKWORM *BOMBYX MORI* L.

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INTRODUCTION

TRANSAMINASE activity is reported to be present in the tissues of silkworm, *Bombyx mori* L.[1-6] The activity is well defined in the silk gland, alimentary canal, muscle, fat body, and hæmolymph. It is reported that the inclusion of micro-quantities of the antibiotic chloromycetin in the diet of the silkworm results in a large increase in the transaminase activity in the hæmolymph and other tissues.[7] Antibiotic inclusion also improves the resistance against diseases like polyhedrosis (grasserie) and Flacherie.[7] Polyhedrosis attacks the silkworm larva at all its developmental stages[8]; it can also be induced by varying the conditions of rearing, like temperature,[9] humidity, nutrition,[10] and feeding of chemicals.[11] Polyhedrosis causes hypoproteinemia.[12] So it is interesting to study the effect of polyhedrosis on some key enzymes in the intermediary metabolism of proteins. This study was carried out to investigate the effect of polyhedrosis on the hæmolymph transaminases of the developed larva of *Bombyx mori* L.

MATERIALS AND METHODS

Larvæ of the silkworm were chosen in their fifth age just before spinning. They were picked up from the mountages when they were ready for spinning cocoons. The healthy larvæ and the grasserie-affected larvæ were collected in separate groups. The larvæ were pierced to bleed into 50 ml. capacity polythene centrifuge tubes placed in an ice-bath. The hæmolymph was centrifuged at 2,000 r.p.m. in an MSE refrigerated centrifuge at 4° C. for 30 min. The clear supernatant of the hæmolymph was dialised in a cellophane bag against several changes of phosphate buffer (pH 7·4) for 48 hr. at 4° C. The dialysed hæmolymph was used as enzyme source.

Isolation of Polyhedra.—The hæmolymph from grasserie-affected worms was centrifuged at 800 r.p.m. for 30 min. at 4° C. and the residue discarded. The supernatant was then recentrifuged at 3,000 r.p.m. for 1 hr. to obtain the residue (Residue I) of the polyhedral crystals and Supernatant I. Residue I was washed repeatedly with water and recentrifuged. Supernatant I was diluted with three times its volume of water to reduce the density of the liquid and centrifuged at 10,000 r.p.m. for 1 hr. which would completely sediment most of the polyhedral particles (Residue II), which were as before washed with water. Residues I and II were combined to provide the polyhedral preparation.

The amino-acids and the keto-acids and other chemicals employed were from commercial sources (BDH or E. Merck).

0·08 M solutions of the amino-acids and keto-acids were prepared in phosphate buffer (pH 7·4, containing 18·2 g. of $Na_2HPO_4.12\,H_2O$ and 1·81 g. KH_2PO_4 per litre). In the case of tryptophan and isoleucine which were difficult to dissolve in the buffer, the amino-acids were first dissolved in minimum amount of dilute alkali and solution made up to 0·08 M with the buffer.

$CdSO_4$ (0·08 M) was prepared in distilled water. Ninhydrin (0·01%) was prepared in acetone. Pyridoxal phosphate (200 μg./ml.) solution was prepared in the buffer.

The incubation mixture consisted of 0·25 ml. each of the amino-acid and keto-acid along with 0·1 ml. of pyridoxal phosphate and 0·25 ml. of the dialised hæmolymph. Control tubes did not contain the enzyme source which was added at the end of the incubation period just prior to stopping the reaction. The tubes were incubated at 37° C. for 1 hr. and then were transferred to boiling water-bath for 5 min.

The reaction mixture was centrifuged at 2,000 r.p.m. for 30 min. at 25° C. The clear supernatants were transferred into different sample tubes.

The supernatants were spotted on Whatman No. 1 paper for chromatography by means of a micro-pipette. Solutions of standard amino-acid were also spotted for reference. The chromatograms were developed either by using *n*-butanol, acetic acid and water (either 2 : 2 : 1 or 4 : 1 : 5) or phenol saturated with water. The chromatograms were dried at room temperature and were dipped in ninhydrin

solution. The spots were developed by heating the chromatogram at 80–85° C. for 3 mm. The coloured spots thus developed were cut out into strips. The strips were fixed between microscope slides and kept in an elution tank and were eluted into graduated tubes. To each tube, 0·05 ml. of $CdSO_4$ solution was added and the resulting colour read at 510 mμ in Beckman B spectrophotometer. The amount of amino-acid (alanine or glutamate) was calculated from a calibration curve set up with corresponding amino-acids. The transaminase activity was expressed as milligrams of amino-acid formed per ml. of the hæmolymph per hr. at 37° C.

The isolated polyhedral bodies were suspended in phosphate buffer (pH 7·4) and used as enzyme source for incubation in separate experiments.

Results

It is observed that the activities of the enzymes glutamate-alanine, and glutamate-aspartate transaminases have increased nearly five times from the normal (Fig. 1). In normal animals, the aspartate-alanine transaminase and glutamate-alanine transaminase activities are extremely low and almost immeasurable. In diseased conditions, their activities increased 15–20 times. Of the several amino-acids tested for amino group donor activity (Table I) to either (*i*) pyruvate, or (*ii*) α-ketoglutarate,

TABLE I

Transaminase activity in the hæmolymph of normal and grasserie-affected larva of Bombyx mori *L.*

Sl. No.	Amino-acid	Keto-acid*	Presence of transaminase activity	
			Normal	Grasserei
1	L-Serine ..	(i)	–	–
		(ii)	–	–
2	DL-Threonine ..	(i)	–	–
		(ii)	–	–
3	L-Lysine ..	(i)	–	–
		(ii)	–	–
4	DL-Tryptophan ..	(i)	–	–
		(ii)	–	–
5	Glycine ..	(i)	–*	–*
		(ii)	–	–
6	DL-Valine ..	(i)	–*	–*
		(ii)	–	–
7	L-Arginine ..	(i)	–*	–
		(ii)	–	–
8	DL-Isoleucine ..	(i)	–	–*
		(ii)	–*	–*
9	DL-Methionine ..	(i)	–*	–*
		(ii)	–	–
10	DL-Phenylalanine ..	(i)	–*	–
		(ii)	–	–
11	DL-Histidine ..	(i)	–	–*
		(ii)	–*	–
12	DL-Alanine ..	(ii)	+	+
13	L-Glutamic acid ..	(i)	–*	+
14	DL-Aspartic acid ..	(i)	–*	+
		(ii)	+	+

* (i) Pyruvate, (ii) α-Ketoglutarate.

Note: – No activity; –* Extremely slight activity (not quantitatively measurable), + Activity measured quantitatively.

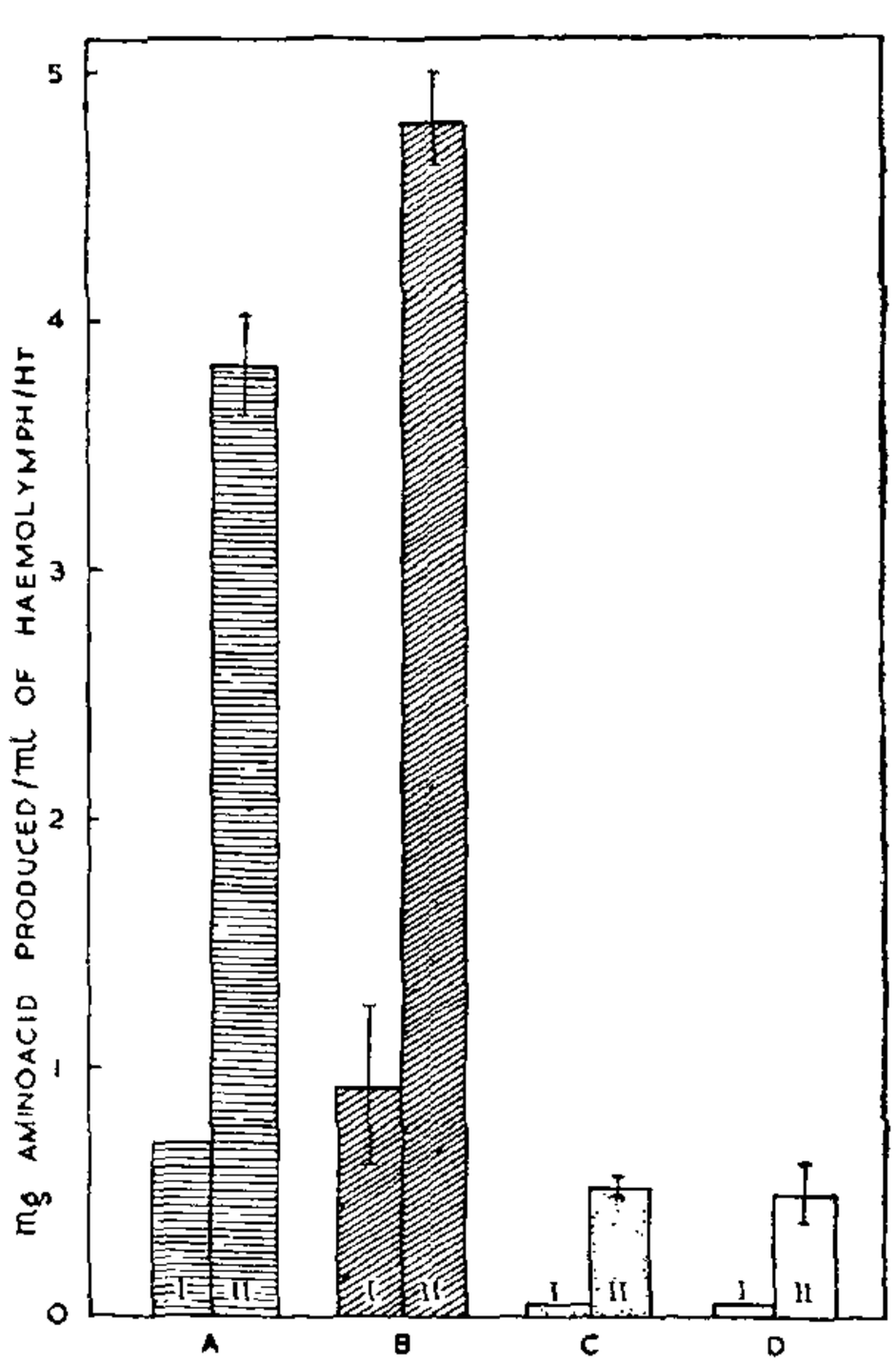

FIG. 1. A—Glutamate-alanine transaminase: (I) Normal, (II) Grasserie; B—Glutamate-aspartate transaminase: (I) Normal. (II) Grasserie; C—Alanine-aspartate transaminase: (I) Normal, (II) Grasserie; D—Glutamate-alanine transaminase; (I) Normal, (II) Grasserie.

L-serine DL-threonine, L-lysine and DL-tryptophan do not appear to show any transfer activity to either pyruvate or α-ketoglutarate while glycine, DL-alanine and DL-methionine have only a trace amount of activity towards pyruvate as detected by chromatogram and none towards α-ketoglutarate. L-arginine and DL-phenylalanine similarly show trace amount of activity only in normal animals and none in grasserie-affected animals. DL-isoleucine, on the other hand, shows transfer activities in trace amounts towards α-ketoglutarate and none towards pyruvate. Only DL-alanine-α-ketoglutarate transaminase, L-glutamic acid-pyruvate transaminase and aspartic acid transami-

nation either with pyruvate or α-ketoglutarate could be measured quantitatively and significant increase in the activity of each of these enzymes has been shown in polyhedrosis (Fig. 1).

Incubation with isolated polyhedral body in place of enzyme showed no transaminase activities for glutamate-aspartate, glutamate-alanine and alanine-aspartate even though these transaminases are abnormally high in the hæmolymph of grasserie-affected larvæ.

Discussion

Polyhedrosis of silkworm *Bombyx mori* L. is a very serious disease which completely destroys the capacity of the worm to produce silk. The disease has been shown to cause abnormal increase in the concentration of glutamic-aspartate transaminase and of glutamic-alanine transaminase activities. The equilibrium in each of these cases is far in the direction of glutamic acid formation. Several other transaminases : (1) serine-alanine ; serine-glutamic acid, (2) threonine-alanine ; threonine-glutamic acid ; (3) lysine-alanine ; lysine-glutamic acid ; and (4) tryptophan-alanine ; tryptophan-glutamic acid are totally absent. The activity of the following transaminases is detectable only in trace amounts either in normal animals (*a*) or grasserie-affected animals (b).

(*a*) Glycine-alanine ; valine-alanine ; arginine-alanine ; isoleucine-glutamic acid ; methionine-alanine and histidine-glutamic acid.
(*b*) Glycine-alanine ; valine-alanine ; isoleucine-glutamic acid ; methionine-alanine and histidine-alanine.

The only other enzyme which shows an abnormal increase in polyhedral attack even though it is present in trace amounts in normal animals is alanine-aspartate transaminase. Since these enzymes are known to play a central role in protein metabolism, it is apparent that polyhedrosis which causes hypoproteinemia in the *noctuide* larva[12] will thus have an increased concentration of some of these enzymes. It is known that the concentration of transaminases increases when the silkworm is fed with micro-quantities of antibiotics like chloromycetin. However, silkworms have been shown to yield more silk when treated with chloromycetin suggesting an increased protein anabolic activity under these conditions. Polyhedrosis, on the other hand, causes hypoproteinemia and, therefore, the increased concentration of these enzymes might suggest increased protein degradative activity in the host, thus facilitating the production of viral proteins and the multiplication of polyhedral viral particles.

Isolated polyhedra do not show any transaminase activities.

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1. Koide, H., Shishido. T., Nagayama, H. and Shimura, K., *J. Agri. Chem. Soc.* (Japan), 1956, **30**, 283.
2. Fukuda, T. and Hayashi, T., *J. Biochem.* (Tokyo), 1958, **45**, 469.
3. —, *Ibid.*, 1957 **44**, 505.
4. Koide, F., Nagayama, H and Shimura, K., *J. Agri. Chem. Soc.* (Japan), 1955, **29**, 987.
5. Bheemeswar, B., *Nature*, 1955, **176**, 555
6. — and Sreenivasaya, M., *Curr. Sci.* (India), 1952, **21**, 253.
7. Shyamala, M. B. and Bhat, J. V., *J. Sci and Ind. Res.* (India), 1955, **14 C**, 97.
8. Hisao Aruga, *Jour. Silkworm T.I.*, 1957, **9** (1-2), 37.
9. Hukuhara, T. and Aruga, H , *J. Sericult. Sci.* (Japan), 1959, **28**, 235.
10. Aruga, H. and Nagashima, E . "On the relationship between incidence of polyhedral diseases and the nutritional conditions during larval stage in the silkwrom *Bombyx mori* L." (In preparation).
11. — and Hukuhara, T., *J. Seri. Sci.* (Japan), 1960, **29**, 44.
12. Mortignoni, M. E. and Milstread, J. E , *J. Insect pathol.*, 1964, **6**, 517.