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Observation on the free amino acids and hydrogen-ion-concentration of male and female *Antheraea proylei* larva

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Abstract
The qualitative variations in the free amino acids of larval haemolymph and the silkglands of male and female *Antheraea proylei* larva have been studied. The larval haemolymph has been found to contain 19 and 20 free amino acids in male and female respectively. Whereas in the silkgland only 16 free amino acids have been detected in both the sexes. The hydrogen-ion-concentration of the larval haemolymph and silkgland in the two sexes has also been observed to be ranging from 5.5. to 6.0 and 6.0 to 6.5 respectively. This has revealed that the haemolymph and silkgland both are rich in free amino acids. The amino acids obtained from food pass into the haemolymph from where most of the amino acids are picked up by silkgland.

Keywords: *Antheraea proylei*, free amino acids, hydrogen-ion-concentration and silkgland

Introduction
The oak-tasar silkworm, *Antheraea proylei* Jolley is an economically important insect. It is a fertile hybrid between *Antheraea pernyi* of China and *Antheraea roylei* of India. The essential components of all the cells in the living organisms are the amino acids which are the building blocks of proteins. Fairly good amount of works have been done on the physiological aspects of the silkworms by many workers like Pratt (1950), Micks and Ellis (1952) [5] Zaidi and Khan (1972) [10], Pant and Unni (1980) [9] in *Philosamia ricini*, Agarwal and Jolly 1981 [6] in *Antheraea mylitta* and Dhinakar *et al.* (1991) [3] in silkgland of *Bombyx mori.* But no work seems to have been done on this insect. Therefore it was thought desirable to undertaken to know the variation on the free amino-acids and hydrogen-ion -concentration of male and female *Antheraea proylei* larva.

Materials and Methods
The experimental insect, full grown healthy larvae were collected from the Regional Tasar Research Station, Mantripukhri, lmphal. The extracts of haemolymph and silkglands were collected with the help of a sterilized syringe after careful dissection. The silkglands were crushed with a mortar and pistle. Both the haemolymph and silkgland extracts were homogenised separately in the small hand homogeniser with a little volume of 80% ethanol solution. They were centrifuged at 15,000 RPM for 15 minutes and supernatants were taken and mixed with three parts by volume of chloroform in a separating funnel and kept in the dark place for about 12 hrs. The samples were used for spotting on the Whatman No.1. chromatographic paper by microcapillary tubes and spots were dried quickly with the help of an electric hair dryer. Two dimensional paper chromatographic procedures adopted by Micks and Ellis (1952) [5] for the qualitative estimation of free amino acids were employed. The identification of the free amino acids was done by the method adopted by Consden *et al.* (1944) [1] by comparing their Rf values with those of the corresponding reference amino acids. Universal indicator method (David, 1927) [4] and paper indicator method (Waterhouse, 1949) [2] were adopted for the estimation of hydrogen-ion-concentration. In case of the universal indicator method the known buffer solutions of different pH ranges were kept in the crucible. A drop of universal indicator is added in each of the cavity and mixed properly. Simultaneously a drop of the fluid to be tested was taken and mixed with the universal indicator. The change in the colour was compared with the colour resulted by the known buffer solution. In case of the paper indicator technique, small pieces of indicator paper were cut and touched to the fluid to be tested and the colour change immediately after touch was noted and compared with the colour range in the indicator paper. Thus, the pH of the gut in both healthy and diseased larvae were determined.

Fig 1: Photograph of full grown (5th instar) larvae of *Antheraea proylei*

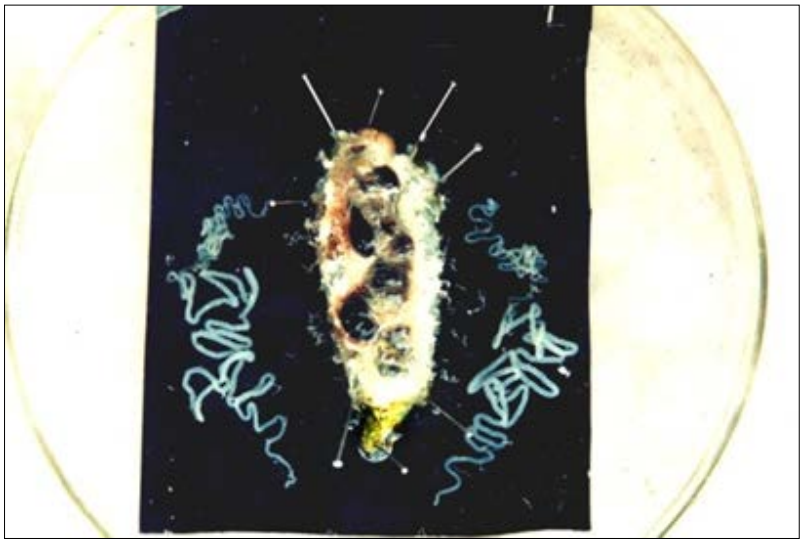

Fig 2: Photograph showing the silkgland of *Antheraea proylei* larvae

Fig 3: Photograph of full grown male larvae showing the sexual marking

Fig 4: Photograph of full grown female larvae showing the sexual marking

Results and Discussion

The study reveals the presence of 19 and 20 free amino acids in the haemolymph of male and female larvae respectively (Table 1). It indicates that the physiological state of the larval haemolymph in both the sexes during their full grown (5^{th} instar) is almost similar as far as qualitative free amino acids are concerned. A similar observation was also made by some workers like Agarwal and Jolly (1981) [6]. Though almost similar in their qualitative presence, some of the free amino acids vary in their concentration in the two sexes as such 13 free amino acids in the male and 11 free amino acids in female occur in higher concentration while 3 free amino acids in male and 7 free amino acids in female occur in moderate concentration. Inspite of this, most of the free amino acids occur almost in similar concentration in both the sexes. It is concluded therefore, that the free amino acid pattern in the larval haemolymph of both the sexes is similar except the absence of ornithine from the male. Thus, the present observation is in agreement with that of Zaidi and khan (1972) [10].

The variations in the free amino acids in Silkgland (Table 1) indicates the presence of 16 free amino acids in silkgland of both the larvae. It appears that the larval silkglands are also similar in their qualitative presence of free amino acids. The free amino acids in silkgland of both the sexes like alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, proline, phenyl- alanine, serine, threorine, lysine, tryptophan, tyrosine and valine are present as the part of these amino acids in food, the gut, the haemolymph, then silkgland and finally to the silken cocoon. No marked sexual dimorphic difference has therefore been observed during the larval stages at least in these free amino acids in the haemolymph and silkgland. The hydrogen ion-concentration of the larval haemolymph and silkglands (Table 2) in male and female has been observed to range from 5.5 to 6.0 and 6.0 to 6.5 respectively. The haemolymph and silkgland of both the sexes are slightly acidic in nature Pant *et al*., (1959) [7]. However, the haemolymph and silkgland in male larvae are more acidic than the female ones.

Table 1: Variation of free amino acids in the haemolymph of male and female larvae of *Antheraea proylei.*

Sl. No.	Amino acids	Haemolymph		Silkglands	
		Male	Female	Male	Female
1.	Alanine	+++	++	++	++
2.	Amino-butyric acid	-	-	-	-
3.	Arginine	+++	+++	++	+
4.	Cysteine	++	+++	-	-
5.	Aspartic acid	+++	+++	+++	+++
6.	Cystine	+	+	-	-
7.	Dihydroxy-phenylalanine	-	-	-	-
8.	Glutamic acid	++	++	+++	+++
9.	Glycine	+++	+++	+++	+++
10.	Histidine	+	+++	+	+
11.	Hydroxyproline	-	-	-	-
12.	lso-leucine	+++	+++	+++	+++
13.	Leucine	+++	+++	+++	+++

14.	Nor-leucine	-	-	-	-
15.	Methionine	+++	++	-	-
16.	Ornithine	-	++	-	-
17.	Proline	+++	+	++	+
18.	Phenyl-alanine	++	++	++	++
19.	Serine	+++	+++	++	++
20.	Threonine	+++	+++	++	++
21.	Lysine	+++	+++	++	+
22.	Tryptophan	+++	++	+	++
23.	Tyrosine	+	++	+++	+++
24.	Valine	+++	+++	+	++
	Total	19	20	16	16

Note 1 The sign + + + represents higher concentration,
+ + moderate,
+ traces and
- absence

Table 2: Hydrogen-ion-concentration in the haemolymph and silkgland of male and female larvae of *Antheraea proylei*

No. of obs.	Hydrogen-ion-concentration			
	Haemolymph		Silkgland	
	Male	Female	Male	Female
1.	5.5	6.0	5.5	6.0
2.	5.5	6.5	5.5	6.5
3.	6.0	6.0	5.5	6.0
4.	6.0	6.0	6.0	6.0
5.	6.0	6.5	6.0	6.5
6.	5.5	6.0	5.5	6.0
7.	5.5	6.0	5.5	6.5
8.	6.0	6.5	5.5	6.0
9.	6.0	6.0	6.0	6.5
10.	5.5	6.5	6.0	6.5
Range	5.5-6.0	6.0-6.5	5.5-6.0	6.0-6.5
Mean	5.75	6.2	5.7	6.25

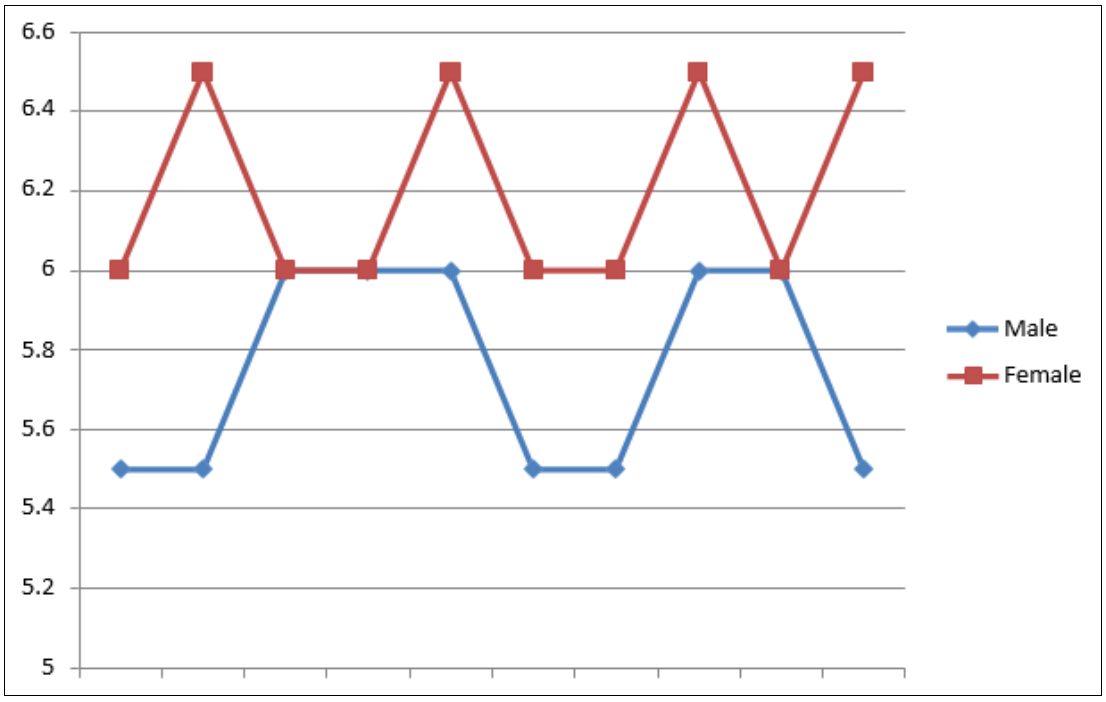

Fig 5: Variation of hydrogen-ion-concentration between the male and female haemolymph of *Antheraea proylei* larva

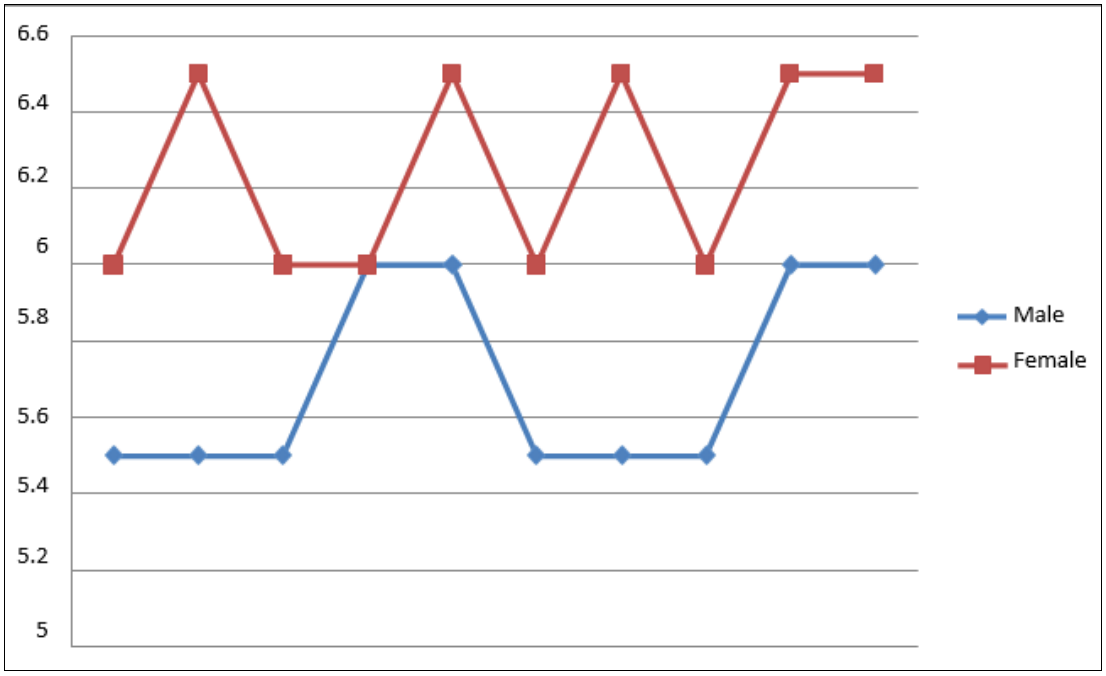

Fig 6: Variation of hydrogen-ion-concentration between the male and female silkgland of *Antheraea proylei* larva

Conclusion

The study has thus revealed that the haemolymph and silkgland both are rich in free amino acids. The amino acids obtained from food pass into the haemolymph from where most of the amino acids are picked up by silkgland through which these larvae secrete the silken fibers forming the cocoon.

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