FREE AMINO ACIDS OF HAEMOLYMPH AND SILK GLAND IN THE DEVELOPING FIFTH INSTAR AND SPINNING LARVA OF *PHILOSAMIA RICINI*

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ABSTRACT

The changes in individual free amino acids in the haemolymph and silk gland of *Philosamia ricini* during fifth instar development and spinning period have been studied. Fifteen amino acids in the silk gland and eighteen in the haemolymph were identified and quantitated. Absence of methionine and other sulphur containing amino acids in the silk gland is noteworthy. Alanine, serine, glycine, lysine, tyrosine, histidine and phenylalanine occur in high concentrations both in silk gland and haemolymph and undergo significant fluctuations during silk biosynthesis.

INTRODUCTION

ONE of the biochemical characteristics of insects in general is the high concentration of free amino acids in their haemolymph as well as in the tissues[1]. The significance of this phenomenon although ascribed to several functions like osmoregulation[2], protein synthesis[3], energy production for flight[4] and cocoon construction[5], has remained an intriguing poser to insect biochemists and one really wonders whether after all, some of the functions attributed to them are unique to the class Insecta!

Silk gland is the site for silk synthesis and is also known to possess extremely high aminotransferase activity[6] and the free amino acids of haemolymph can be taken by other tissues of the growing silkworm including silk gland when it accomplishes the biosynthesis of silk.

Notwithstanding the knowledge of the aforementioned facts, literature does not reveal any definite relationship between amino acid pool of silk gland and haemolymph either individually or collectively during the process of silk synthesis. With a view to elucidating this, the individual free amino acids of the two tissues during development of the fifth instar larva as well as during silk spinning have been studied and correlated.

MATERIALS AND METHODS

Philosamia ricini larvae were reared in the laboratory as described earlier[7]. Haemolymph from the fifth instar larva was collected in pre-cooled beakers as described earlier[8] and known volumes thereof (0·2–0·5 ml) were directly pipetted into centrifuge tubes containing ethanol (5 ml, 75% v/v) well stirred and kept for about 20 min at room temperature and then centrifuged. The precipitated protein was washed several times with ethanol (75% v/v), till the supernatant did not react with ninhydrin. The pooled extract of amino acids was evaporated to dryness in partial vacuum and dissolved in a known volume of distilled water (1·0 ml) and employed for amino acid estimation by Lee and Takahashi's method[9]. Amino acids were identified with special spray reagents[10].

For amino acid assays of the silk gland, larvae were dissected longitudinally from the dorsal side in Bodenstein's insect Ringer solution and the long, thread-like slender silk glands were carefully detached from the connective tissue. An indirect method had to be resorted to, due to the hardening of the tissue when brought into contact with ethanol for extraction. The free amino acids were therefore extracted from a homogenate (10% w/v) prepared in ice-cold glass-distilled water employing an all-glass Potter-Elvehjem type of homogeniser. The homogenate was filtered through nylon cloth and known volumes (2·5 ml–5·0 ml) of the filtrate in triplicate were used for amino acid extraction. The extraction and assay procedures employed for amino acids were the same as described above for those of haemolymph.

RESULTS AND DISCUSSION

The typical U-shaped variation patterns of glycine (Fig. 5) and tyrosine (Fig. 15) of silk gland origin during spinning period (0–24 hr), is noteworthy. Larval haemolymph maintains all through the fifth instar development, a low concentration of alanine, glutamic acid and proline whereas arginine, cystine. histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and tyrosine reveal higher concentrations during days 3–5. Despite this, all the amino acids irrespective of their accumulation or utilization during development re-attain their initial level nearabout pupation. In contradistinction, glycine, valine, serine and tryptophan steadily concentrated in the haemolymph of the developing larva but precipitously declined during spinning. Aspartic and glutamic acids were continuously utilized by the developing larva but the spinning insect accumulated them.

The silk gland appears to withdraw appreciable quantities of isoleucine, leucine (Fig. 7), lysine (Fig. 8), phenylalanine (Fig. 10), threonine (Fig. 13) and valine

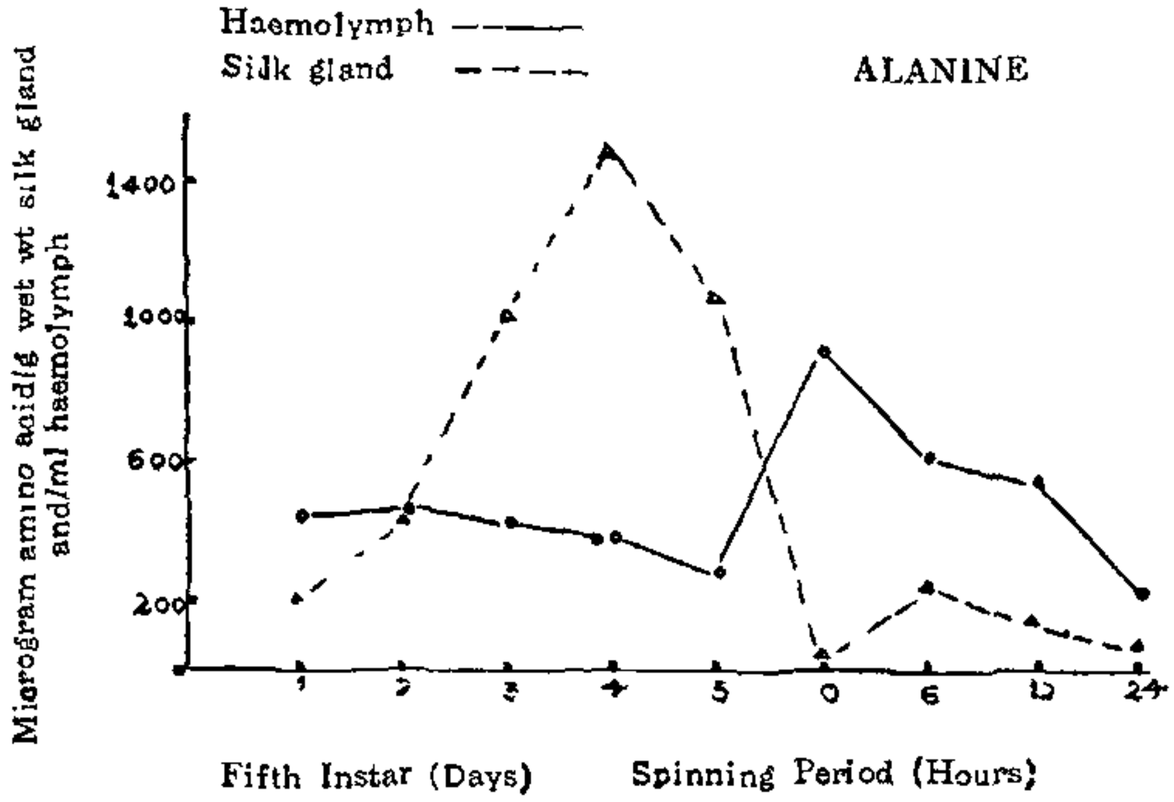
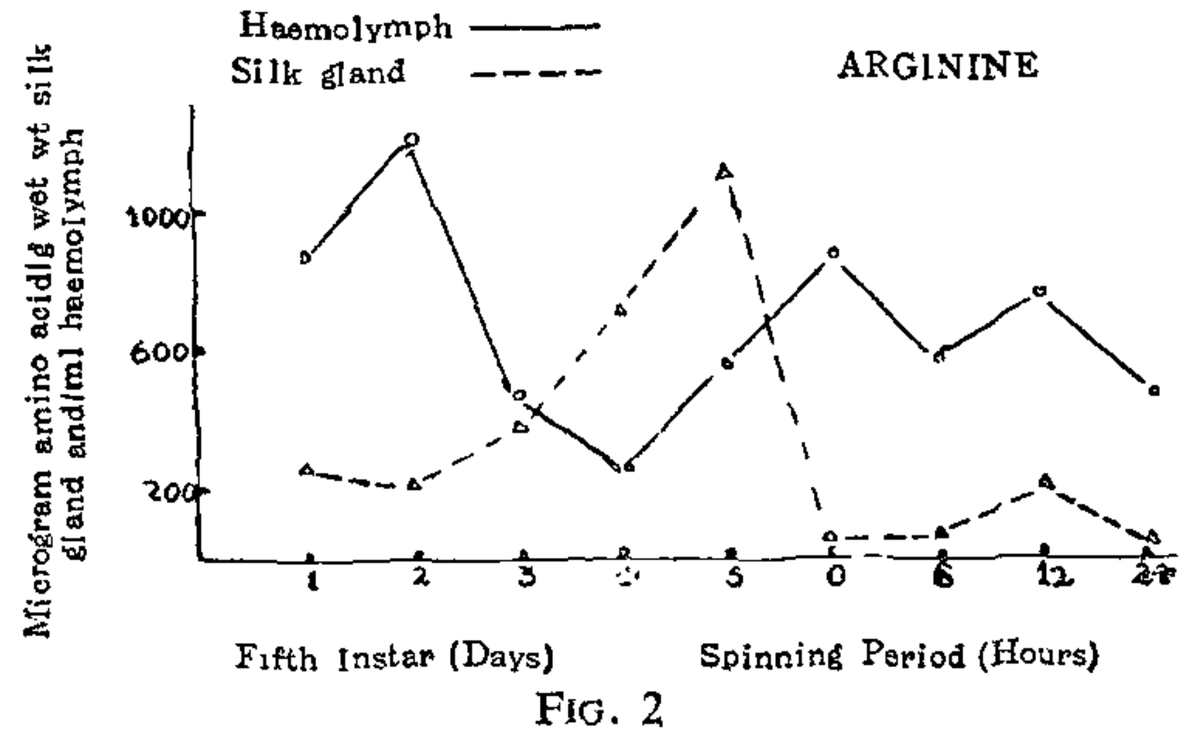


FIG. 1



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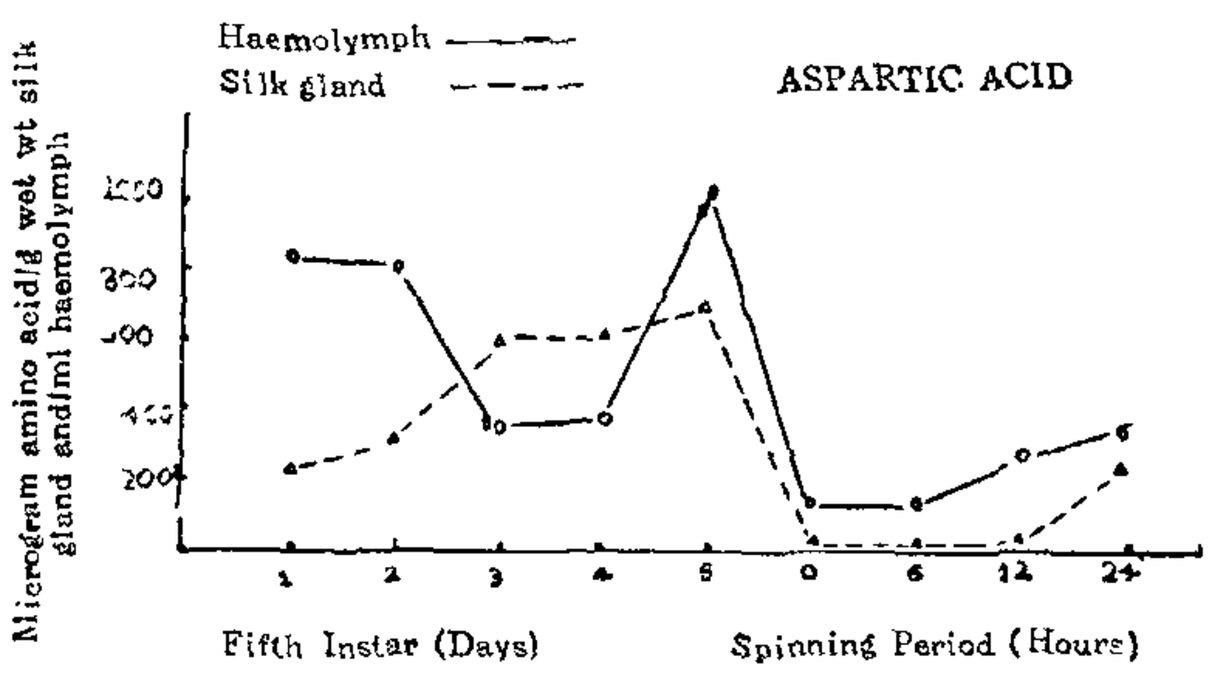

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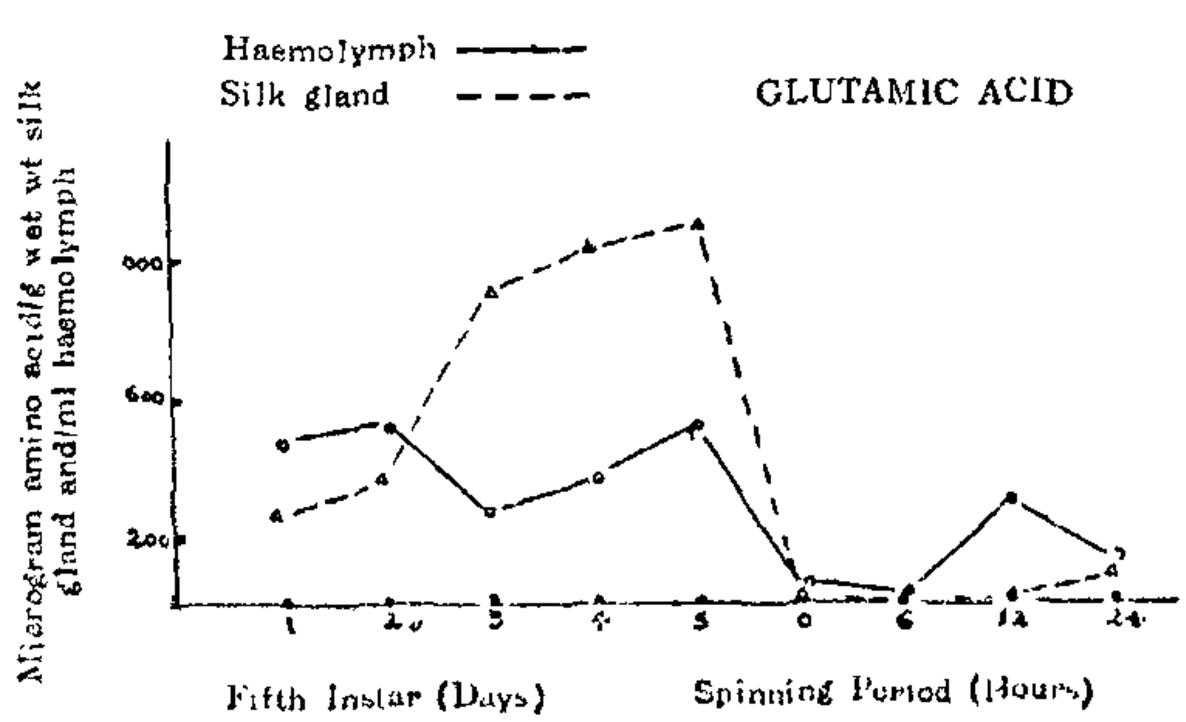

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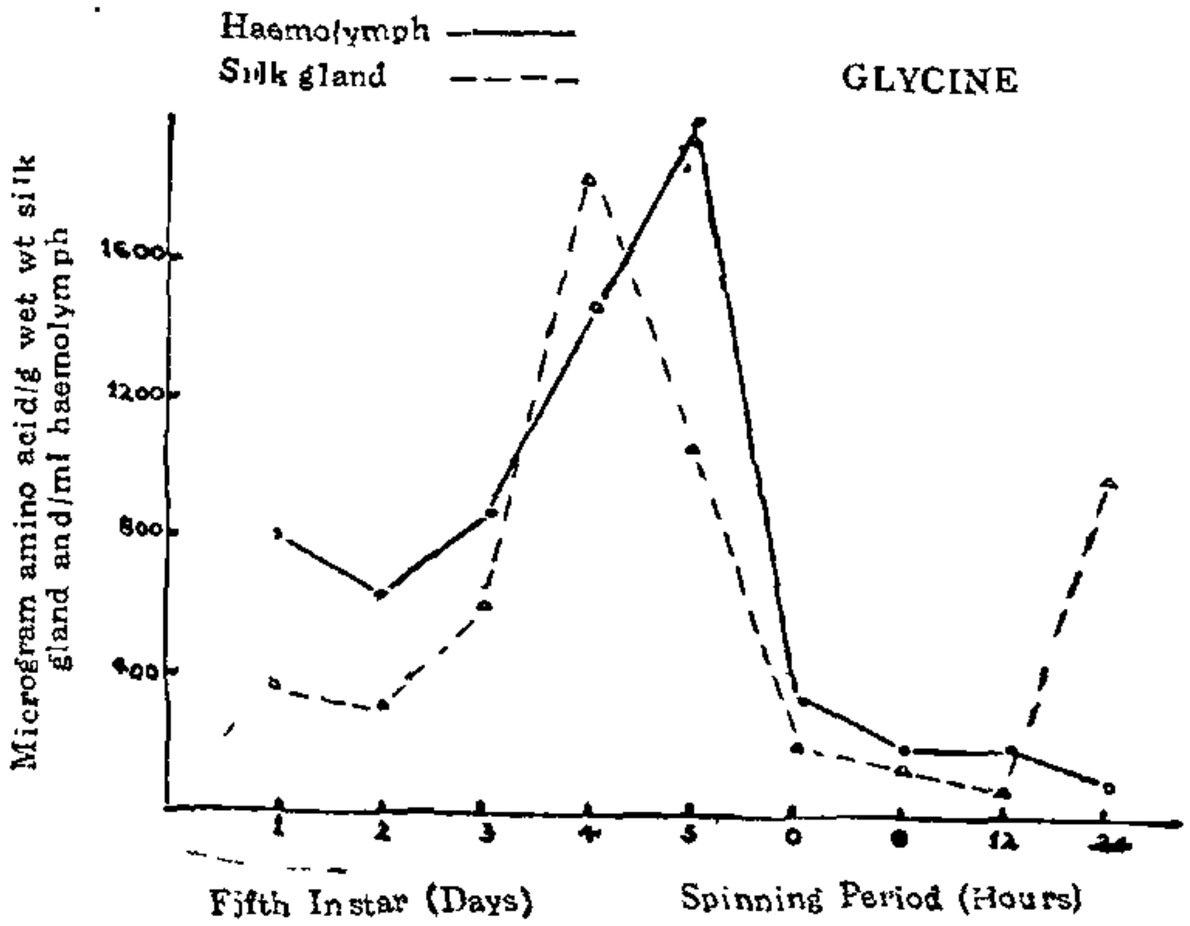

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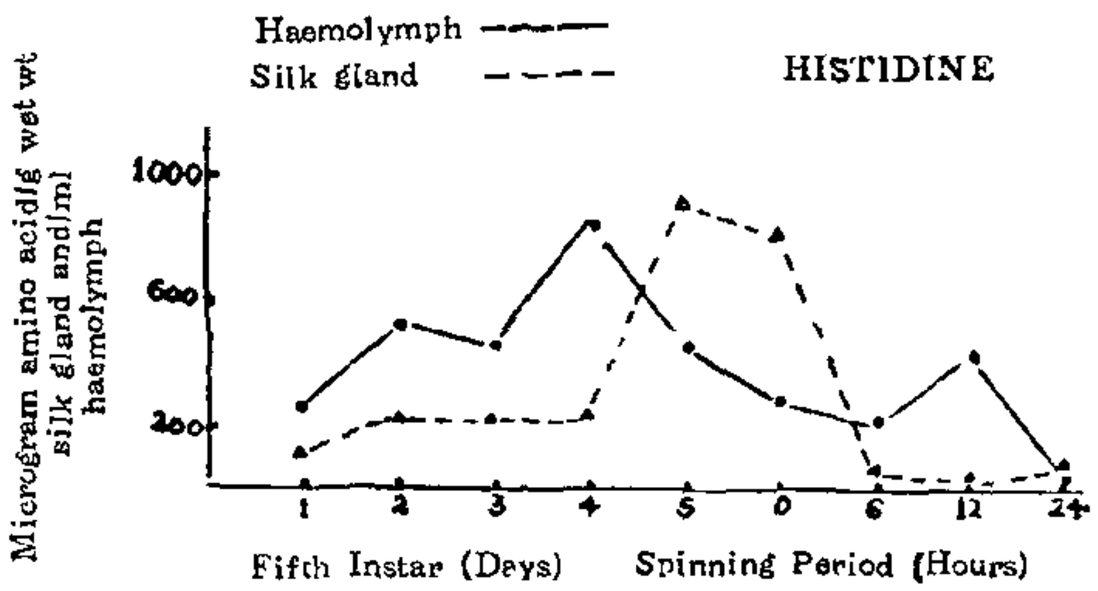

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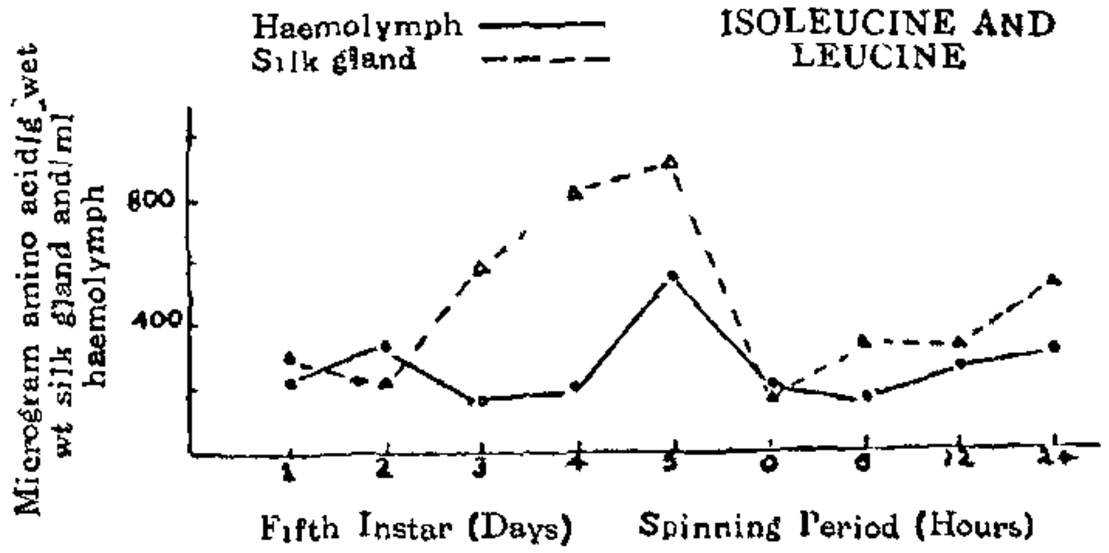

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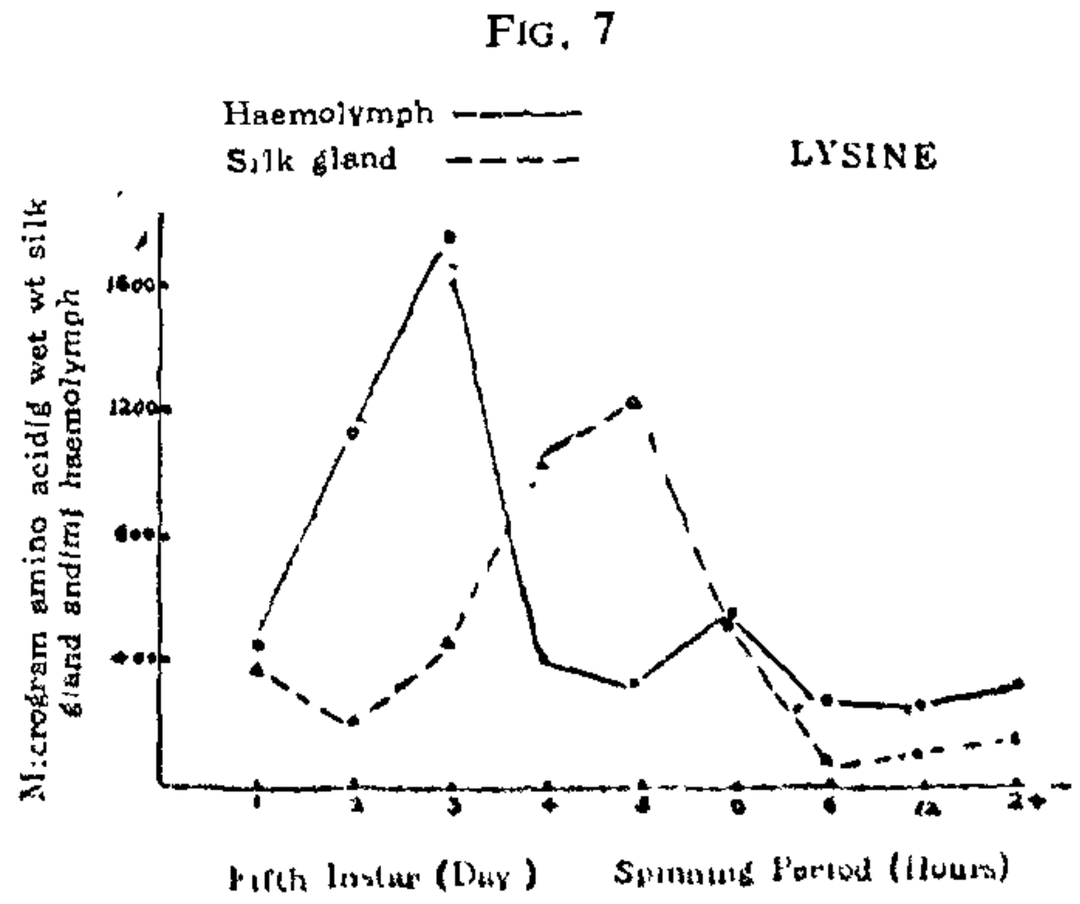

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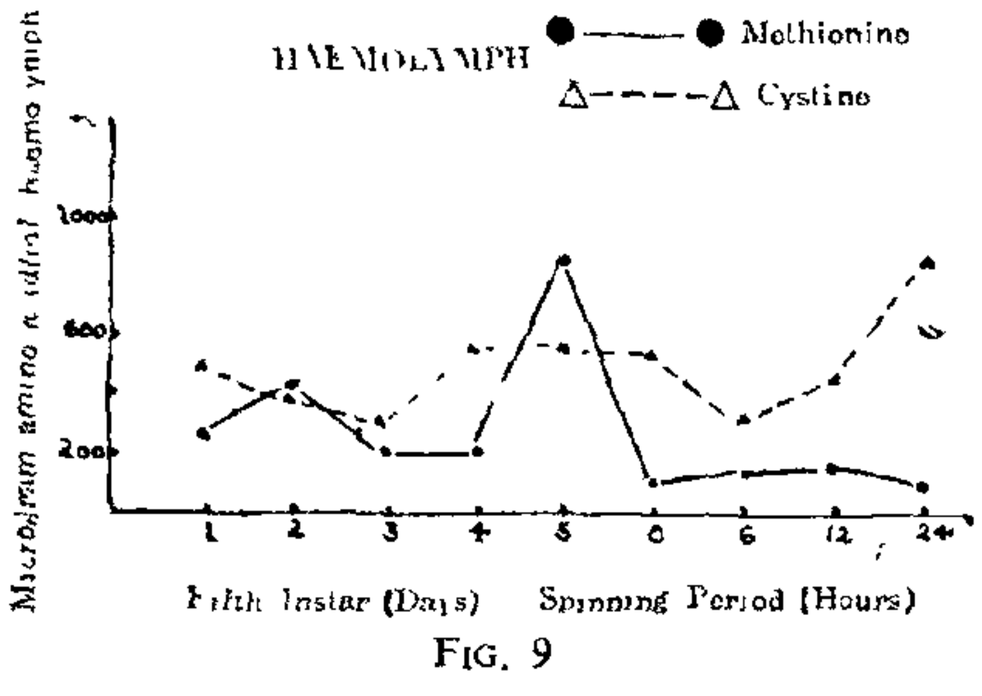

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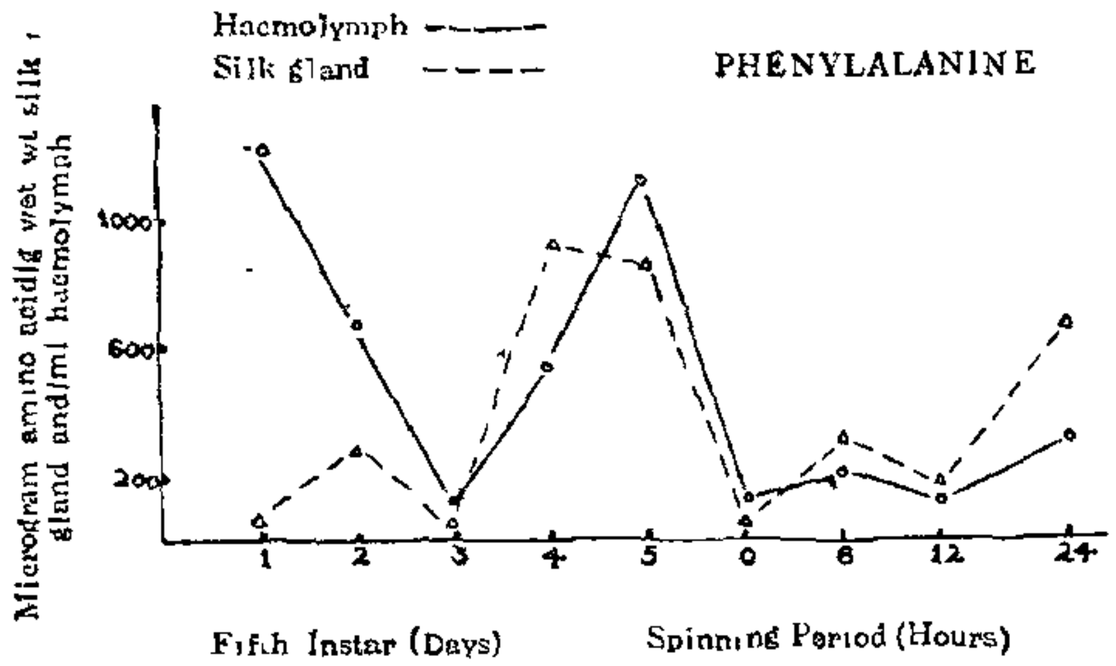

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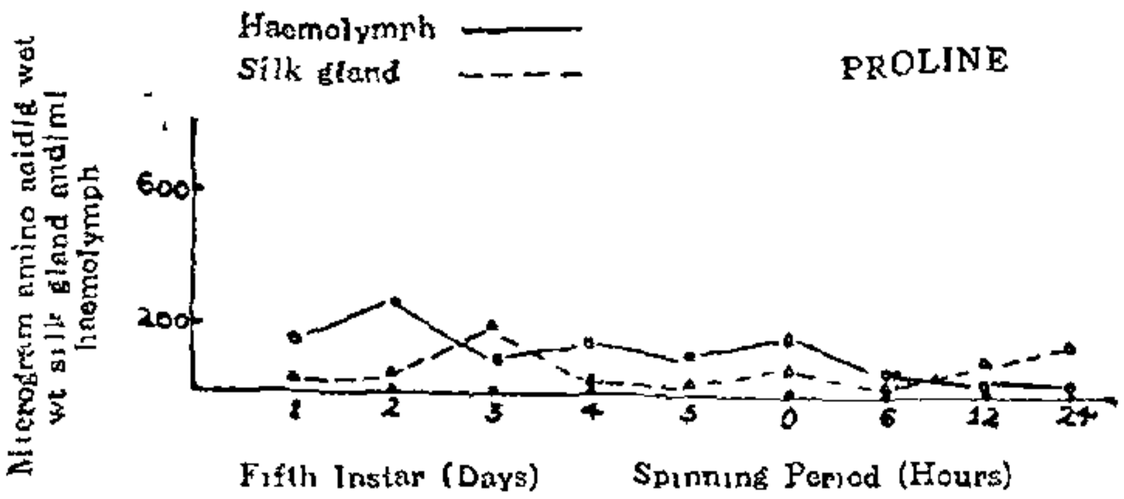

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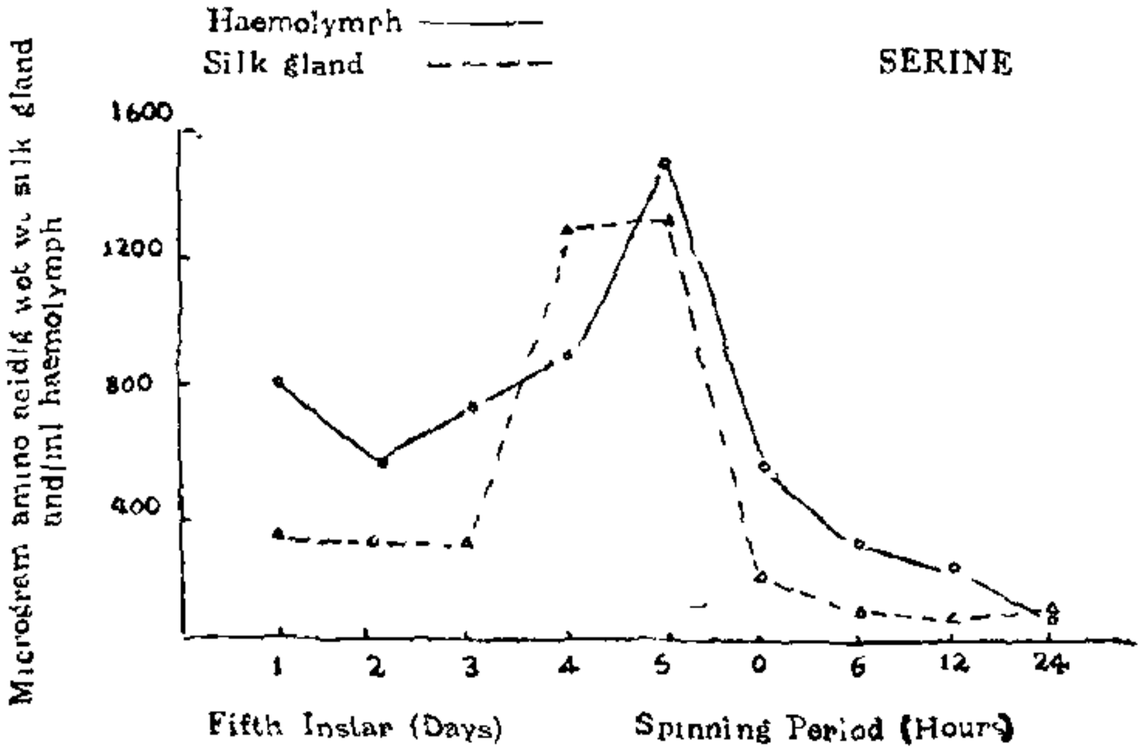

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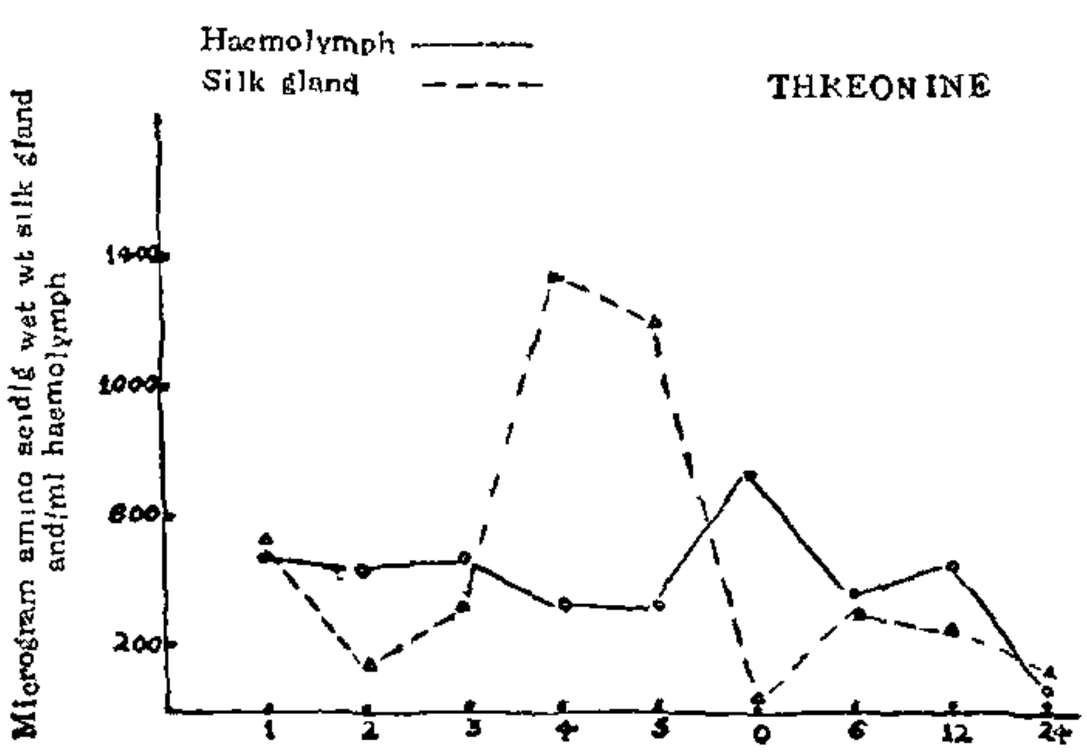

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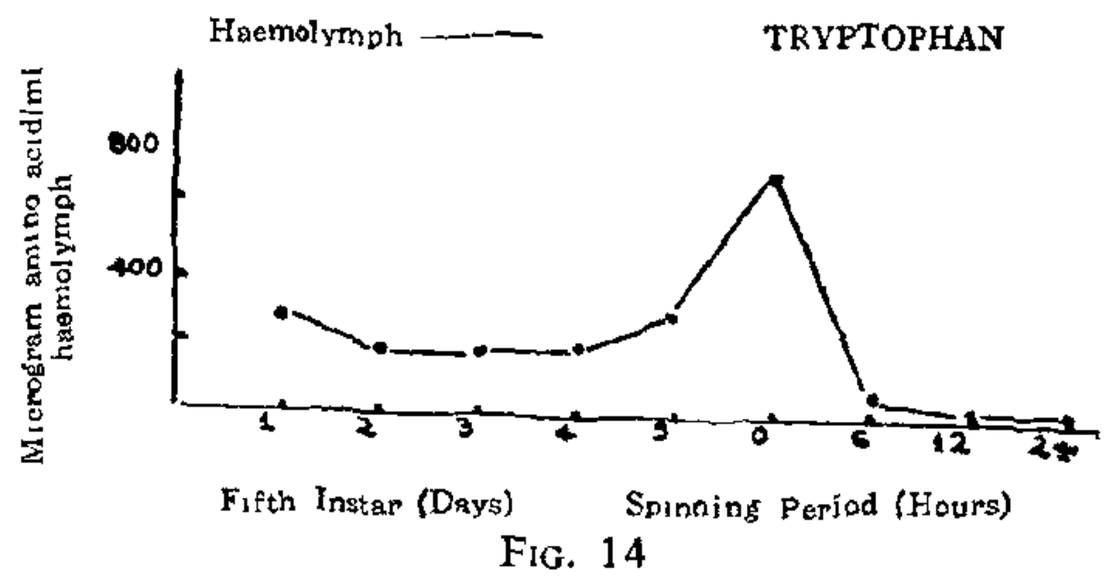

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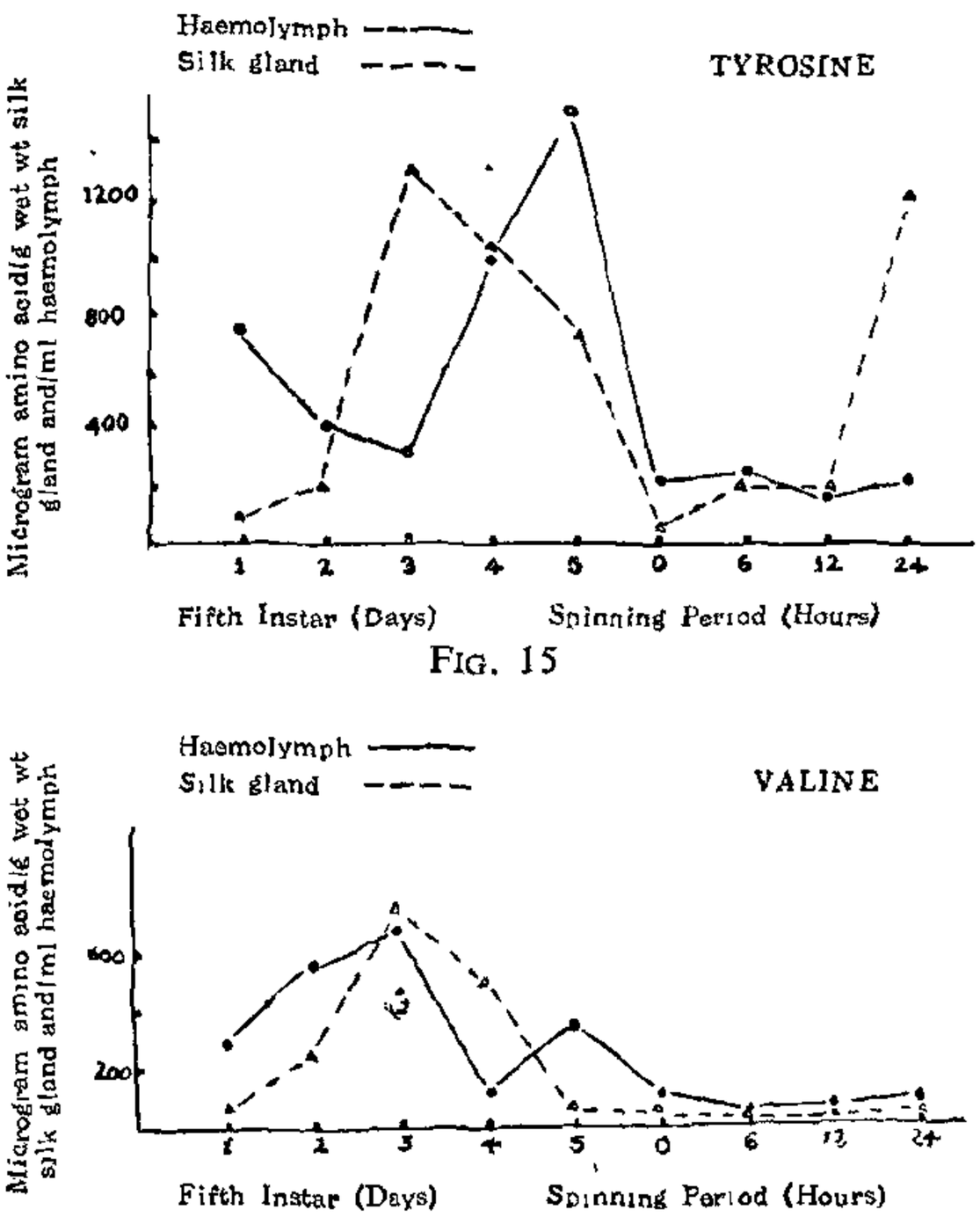

FIG. 15

FIG. 16

(Fig. 16) from the haemolymph as evinced by the patterns traced by them during larval development while aspartic (Fig. 3) and glutamic acids (Fig. 4), glycine (Fig. 5) and tyrosine (Fig. 15) accumulate in significant quantities in both the tissues during growth and utilized for spinning. Absence of sulphur containing amino acids and tryptophan (Fig. 14) and low concentration of proline (Fig. 11) and valine (Fig. 16) all through development in silk gland is interesting. The low concentration of these amino acids is reflected in the silk fibres of *Philosamia ricini*[11].

Before the onset of spinning, practically all the amino acids accumulate in appreciable quantities in both the tissues, probably preparative to silk synthesis and once the process commences they all get significantly depleted in the haemolymph and thence maintain a low concentration till 12 hr after commencement of spinning. The relatively higher concentrations of these amino acids observed all along in the haemolymph, as compared with their concentrations in the silk gland, suggests them to be the source for silk biosynthesis.

Glycine maintains a low concentration during days 1–3 both in the larval silk gland and haemolymph but increases thereafter till commencement of spinning (Fig. 5). This enhanced level of glycine during late larval development could be attributed to its synthesis by transamination mediated by glyoxylic acid and alanine a reaction which occurs at a higher rate in silk worms[12].

Serine follows more or less the same trend of variation in both the tissues during early and late fifth instar development (Fig. 12) and registers depleted concentration during spinning, suggesting its incorporation in silk protein. Alanine, with an initial low concentration, gets accumulated during fifth instar development and decreases during spinning (Fig. 1). Presence of high concentration of alanine in fibroin and of serine in sericin of *Bombyx mori*[13] and *Philosamia ricini* silk fibres[11] confirms the veracity of the above statement.

Presence of a high concentration of aspartic (Fig. 3) and glutamic acids (Fig. 4) both in the silk gland and haemolymph during larval development and their variation profile stress the importance of transamination reaction[6] in the process of growth, development and silk synthesis. Further, radioisotopic studies have also shown that both aspartate and glutamate are mainly utilized by the silk gland for biosynthesis of alanine and silk protein fibroin[14].

Phenylalanine, a precursor of tyrosine, increases gradually and accumulates in large quantities on days 4 and 5 in the silk gland (Fig. 10). Likewise, in the haemolymph also it registers two high peaks on days 1 and 5. Comparatively low concentration of phenylalanine in both the tissues prior to spinning is suggestive of its utilization in silk protein synthesis. High concentration of tyrosine in haemolymph during larval development (Fig. 15) strengthens the findings of Raghavan and Nadkarni in the rice moth[15].

Occurrence of high concentration of arginine in the haemolymph observed before commencement of spinning (Fig. 2) has been implicated in morphological changes[16]. The initial high concentration of histidine gets depleted after spinning for 6 hr in both the tissues (Fig. 6). This is in agreement with the observation of Levenbook[17] and Duchateau-Bosson[18].

The relatively high concentrations of amino acids in the haemolymph all through fifth instar development and spinning period (as compared with the silk gland) confirm the findings of Chitra and Sridhara in *Bombyx mori*[19] that the silk gland withdraws amino acids for silk synthesis from the pool of amino acids of haemolymph origin. In *P. ricini* silk spinning lasts for 24 hr of which the initial 0–12 hr is the most active period. The small upheavals revealed by the haemolymph amino acids during spinning could be attributed to their synthesis *via* the high amino transferase activity[6] and the breakdown of protein[20].

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1. Florkin, M., *L'evolution Biochimique, Paris* Masson, 1944.
2. Bishop, G. H., Briggs, A. P. and Ronzoni, E., *J. Biol. Chem.*, 1925, **66**, 77.
3. Buck, J. B., In *Insect Physiol.* (ed. by Roeder, K. D.), John Wiley and Sons, Inc., N.Y., 1953, p. 147.
4. Sacktor, B., *Arch. Biochem. Biophys.*, 1953, **45**, 349.
5. Wyatt, G. R., *Ann. Rev. Ent.*, 1961, **6**, 75.
6. Pant, R. and Unni, B. G., *Indian J. Exp. Biol.*, 1978, **16**, 705.
7. — and Lacy, P. S., *Indian J. Biochem.*, 1968, **5**, 13.
8. — and Gupta, D. K., *Curr. Sci.*, 1977, **46**, 549.
9. Lee, V. P. and Takahashi, T., *Anal. Biochem.*, 1966, **14**, 71.
10. Pant, R. and Agrawal, H. C., *J. Insect Physiol.*, 1964, **10**, 443.
11. — and Unni, B. G., *Curr. Sci.*, 1978, **47**, 681.
12. Bheemeswar, B., *Sym. 4th Int. Cong. Biochem.*, 1958, **12**, 78.
13. Lucas, F., and Rudall, K. M., In *Comprehensive Biochem.*, 1968, **26B**, 475.
14. Fukuda, T., Hayashi, T. and Shimura, K., *J. Biochem. (Tokyo)*, 1955, **27**, 14
15. Raghavan, K. G. and Nadkarni, G. M., *Indian J. Exp. Biol.*, 1977, **15**, 558.
16. Pant, R. and Verma, R., *Indian J. Biochem.*, 1969, **6**, 45.
17. Levenbook, L. and Dinamarca, M. I., *J. Insect Physiol.*, 1966, **12**, 1343.
18. Duchateau-Bosson Gh., Florkin, M. and Jeuniaux, Ch., *Arch. Int. Physiol. Biochem.*, 1960, **68**, 327.
19. Chitra, C. and Sridhara, S., *Curr. Sci.*, 1972, **41**, 52.
20. Pant, R. and Morris, I. D., *Indian J. Biochem. Biophys.* 1972, **9**, 199.