

AMINO ACIDS IN THE SILK GLANDS OF THE SILKWORM *BOMBYX MORI* L.

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INSECT blood is known to carry unusually high amounts of free amino acids which in some species may be sixty times or more than that in human blood¹. Insect tissues also contain large amounts of free amino acids². Silkworm blood is rich in glutamine, histidine, lysine, serine and glycine³. However, the silk fibroin which is produced by the silk gland differs from other proteins in its high proportion of glycine, alanine and tyrosine⁴. Moreover, the silk gland has been shown to synthesize this protein at a rate faster than the synthesis of even liver proteins. The supply of amino acids needed for this is derived from the blood⁵⁻⁶.

Considerable attention had been given in the past to the mechanism of silk synthesis in this insect. Prudhomme and Chavancy⁷ have shown, for instance, that the endogenous amino acids in the gland have an effect on the uptake and incorporation of externally added amino acids. It is not definite, however, whether or not the passage through the endogenous pool is obligatory for incorporation of external amino acid into the fibroin, though it is known that the amino acid composition of the fibroin varies significantly from that of the haemolymph³⁻⁴. While studying the biochemistry of the silk gland it was always assumed that the concentration of free amino acids found in the silk gland must be the optimum required for silk synthesis, whether or not it is similar to that of haemolymph or fibroin. To ascertain this assumption the total amino acid content and the amounts of glycine, serine, alanine, tyrosine and phenylalanine were determined on all days during V instar.

MATERIALS AND METHODS

Extraction of free amino acids from the silk gland.—Cross breed silkworms *Bombyx mori* L. (Mysore × C. nichi) obtained from the state grainage were maintained in the laboratory during the V instar. Experiments were carried out with V instar larvae. Larvae were anaesthetized with ether and dissected on a

cork board and the posterior portions of the silk glands were separated from the reservoir and were rinsed twice in the ice-cold phosphate buffer (0.06 M, pH 7.0) and transferred into a tube containing 8 to 10 ml of 75% ethanol. As many as 40 glands during the first two days and 20 glands during subsequent days were used in each experiment. Endogenous amino acids from the pooled sample of each day were extracted by keeping the tubes in a boiling water-bath for 5 min. The extract was transferred to a flask and the residue was re-extracted twice in a similar manner. The extracts were pooled, the solvent was removed under suction, and the residue was taken in a known volume of 50% ethanol. Insoluble residue, if any, was removed by centrifugation. The total amino acid content was then determined by the method of Rosen⁸ and the individual amino acids were separated by paper chromatographic method⁹. Glycine and serine from the mixture were separated using phenol: water system (4:1 v/v); alanine, tyrosine and phenylalanine were separated using *n*-butanol: acetic acid: water system (4:1:5 v/v). Amino-acids were eluted from the paper strip using 50% ethanol and then estimated individually by Rosen's method⁸. The free amino acids in the silk gland were also estimated in an automatic amino acid analyzer by the method of Moore and Stein¹⁰. The dry weight of the gland was determined after drying six glands overnight at 60° C.

RESULTS AND DISCUSSION

The results presented in Fig. 1 show that the total amino acid content increased from day 1 to the day 7 during V instar. During this time the wet weight of the posterior portion of the gland alone increased in size from 6.0 mg to 52.0 mg in this batch. The dry weight of the gland, however, always ranged between 15 and 16% of the wet weight. Calculated on the basis of unit dry or wet weight, the total amino-acids remained almost the same during V instar (Fig. 1 and Table I). It is known that during V instar the enormous increase in the size of

TABLE I

Variation in total amino acid content in the silk gland during V instar

Day	Weight of the silk gland in mg		Total amino-acid content in μ g		
	Wet	Dry	Per gland	Per mg wet weight of the gland	Per mg dry weight of the gland
2	6.30	0.93	15.0	2.38	16.13
3	15.62	2.34	39.3	2.52	16.80
4	30.90	4.64	80.7	2.61	17.40
5	38.89	5.80	106.6	2.74	18.38
6	48.67	7.29	134.7	2.77	18.48
7	52.10	7.80	140.0	2.69	17.95
8	26.09	3.91	67.5	2.59	17.26

the silk gland is due to the increase in cell material only and not their number^{11,12}.

It is evident from Fig. 1 and Table II that the concentrations of glycine, alanine, serine, phenylalanine and tyrosine follow clearly the pattern exhibited by the total amino acid content. It is interesting to note that the percentage concentration of the five amino acids nearly remained constant during V instar and it showed no correlation whatsoever with the amino acid composition of haemolymph or fibroin (Table III). Notwithstanding the fact that these five amino acids constitute more than 90% of the fibroin, in the silk gland they make

TABLE II

Variation and percentage composition of some amino acids (glycine, alanine, serine, tyrosine, and phenylalanine) in the silk glands during V instar

Day	Glycine				Alanine				Serine			
	a	b	c	d	a	b	c	d	a	b	c	d
2	0.570	3.8	0.090	0.613	0.600	4.0	0.095	0.645	2.025	13.5	0.322	2.178
3	1.572	4.0	0.101	0.672	1.651	4.2	0.106	0.706	5.070	12.9	0.325	2.167
4	2.905	3.6	0.094	0.626	3.712	4.6	0.120	0.800	11.300	14.0	0.366	2.415
5	4.157	3.9	0.107	0.717	5.223	4.9	0.131	0.901	14.710	13.8	0.378	2.536
6	4.715	3.5	0.097	0.647	5.657	4.2	0.116	0.776	19.130	14.2	0.393	2.624
7	5.320	3.8	0.102	0.682	6.440	4.6	0.124	0.826	21.130	15.1	0.406	2.709
8	2.760	4.0	0.104	0.691	2.903	4.3	0.111	0.742	9.924	14.7	0.380	2.538

Day	Tyrosine				Phenylalanine			
	a	b	c	d	a	b	c	d
2	0.375	2.5	0.060	0.403	4.650	31.0	0.738	5.000
3	0.785	2.0	0.050	0.336	11.987	30.5	0.768	5.125
4	1.453	1.8	0.047	0.313	23.807	29.5	0.771	5.133
5	2.345	2.2	0.060	0.404	34.112	32.0	0.817	5.882
6	3.502	2.6	0.072	0.480	37.851	28.1	0.778	5.192
7	3.920	2.8	0.075	0.503	41.860	29.9	0.804	5.367
8	1.620	2.4	0.062	0.414	21.465	31.8	0.822	5.491

a = μ g per gland, b = %, c = μ g per mg wet weight of the gland, d = μ g per mg dry weight of the gland.

TABLE III

Percentage composition of some amino acids in the silkworm haemolymph, silk gland and silk fibroin

Amino-acid	Silkworm haemolymph		Silk gland		Silk fibroin
Glycine ..	4.6 ^a	2.0 ^b	2.3 ^d	3.8 ^c	43.7 ^e
Alanine ..	4.6	2.7	2.2	4.4	28.8
Serine ..	10.1	7.3	10.4	14.0	11.9
Tyrosine ..	3.5	3.2	2.5	2.3	5.1
Phenylalanine	1.1	1.5	34.9	30.4	0.6

Experimental details under Fig. 11.9.

^a Reference No. 3, ^b Reference No. 6, ^c Reference No. 4, ^d and ^e Larvae from different batches, ^d Estimated in an automatic amino acid analyzer, ^e Estimated by paper chromatographic method (Reference Nos. 9, 8).

upto only about 53% of the total (Table IV). However, it is known that all the tyrosine in the fibroin is derived from phenylalanine¹³ inasmuch as the tyrosine content in the silk gland is very low as compared to that of phenylalanine. It is reasonable to assume therefore that while the conversion of phenylalanine to tyrosine takes place, replenishing of phenylalanine can occur from the haemolymph only.

Earlier report¹² indicates that fibroin is synthesized during the later stages of V instar whereas enlargement of the gland and the increase in the RNA content take place in the earlier half of the V instar and before the onset of silk synthesis. In fact, the amino acid content as well as the composition show varia-

cons during this stage¹⁴. While there is thus an increase in the total amino acid content in the gland, the relative proportion of the amino acids remains nearly constant throughout V instar even as the percentage of the composition of the amino acids per unit weight of the tissue remains constant. Hence it is inferred that this percentage of amino acids found in the gland is essential and optimum for protein synthesis by this tissue provided the passage of amino acids through the pool is obligatory.

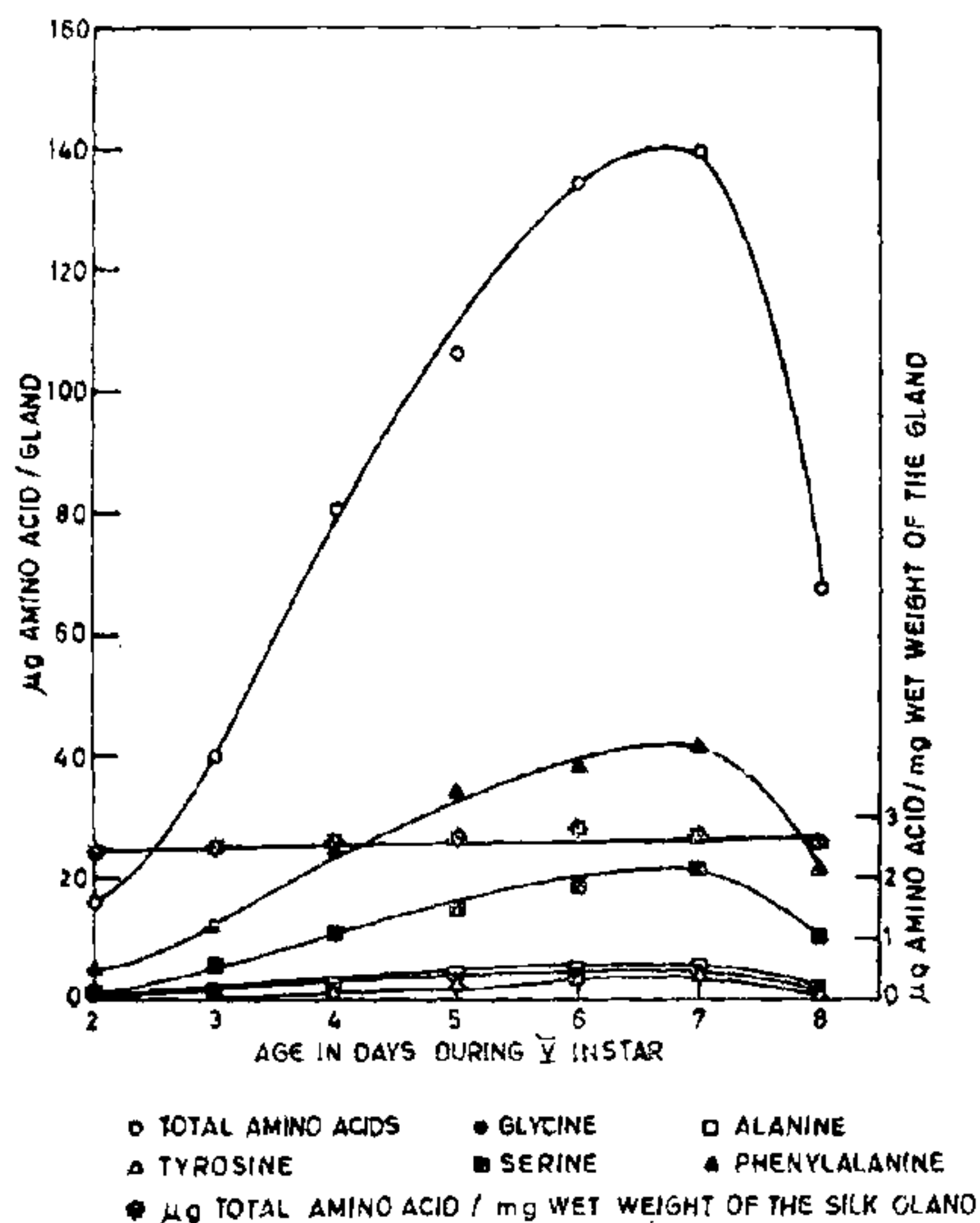


FIG. 1. Variation in total and individual amino acids in the silk gland during V instar.

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TABLE IV
Free amino acids in the silk gland

Amino acid	Quantity per gland		% of free amino acid
	µM	µg	
Lysine	0.039	7.13	2.70
Histidine	0.048	9.15	3.47
Arginine	trace	trace	trace
Aspartic acid	0.132	17.57	6.63
Threonine	0.099	11.79	4.47
Serine	0.261	27.44	10.40
Glutamic acid	0.360	52.95	20.06
Proline	0.012	1.38	0.52
Glycine	0.081	6.08	2.30
Alanine	0.066	5.88	2.23
Cystine	trace	trace	trace
Valine	0.087	10.20	3.87
Methionine	trace	trace	trace
Isoleucine	0.039	5.12	1.94
Leucine	0.066	8.66	3.28
Tyrosine	0.036	6.52	2.47
Phenylalanine	0.558	92.19	34.90
Tryptophan	0.009	1.84	0.70

Determined on the 5th day of V instar in an automatic amino acid analyser.

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