137. Amino Acid Nutrition of the Silkworm, *Bombyx mori*

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Breeding of the silkworm, *Bombyx mori* L., has been carried out with the aim of growing healthy larvae and producing more silk for the practical use. Many studies on amino acid metabolism in this insect have been concerned with the synthesis of silk protein. There are another series of studies on amino acid metabolism pertaining to pigment formation. Data are also available on the ingestion and utilization of amino acids for larval growth and silk production and on the accumulation of free amino acids in hemolymph of silkgland-ectomized larvae. It goes without saying that amino acid nutrition of the silkworm is closely related to the synthesis of silk protein as well as to the growth of silkglands. These studies have in part dealt with the nutritional aspects of amino acids, but information on absolute amino acid requirements has been inconclusive. A method using artificial diets for the analysis of the amino acid requirements of the silkworm has been adopted rather recently.²²⁾ In these studies so-called synthetic diet, or amino-acid diet,²⁵⁾ or meridic diet,⁶⁾ containing L-amino acids as a nitrogen source, was used (Table I). Such a diet ensures larval growth and cocoon-spinning, although it is inferior to a diet containing soybean meal.

Nutritional requirements for amino acids are deeply connected with their metabolic function. Recently, increased attention has focussed on gaining an understanding of amino acid nutrition of the silkworm on the biochemical or metabolic level.³⁰⁾ With due regard to the nutritional role and metabolic function of amino acids in the silkworm, the present author came to a conclusion that L-amino acids are classifiable into five general groups, as follows.

1. Essential amino acids (group-1). *Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.*

Requirements for these ten amino acids were determined by deleting them singly from the diet.^{1),24)} In each instar larvae failed to grow in the absence of the respective amino acid.¹⁹⁾ Apparently, they seem to be hardly synthesized from other amino acids or simpler precursors, or the quantity which is synthesized by the larva is entirely inadequate for supporting growth. It cannot be concluded

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Table I. One of the examples of the composition
of synthetic diet used for the study of amino
acid nutrition of the silkworm

Substance	Dry diet in %
Agar	12.0
Potato starch	11.0
Sucrose	13.5
Amino acid mixture ¹⁾	21.0
β -Sitosterol	0.5
Soybean oil, refined	3.0
Salt mixture, Wesson's	4.0
CaCO ₃	0.4
K ₂ HPO ₄	1.0
Ascorbic acid	2.0
Cellulose powder	31.0
Citric acid	0.4
Morin	0.2
Total	100.0
Vitamin B mixture ²⁾	Added
Antiseptic ³⁾	Added
Dist. water	3 ml/g of dry diet

- 1) L-Amino acids, parts by weight: Arginine-HCl, 6; histidine, 3; isoleucine, 5; leucine, 8; lysine-HCl, 6; methionine, 3; phenylalanine, 6; threonine, 5; tryptophan, 3; valine, 6; proline, 4; K-aspartate, 16; K-glutamate, 14; alanine, 5; cystine, 1; glycine, 3; serine, 5; and tyrosine, 1.
- 2) Vitamins μ g per g of the dry diet: Biotin, 2; choline chloride, 1500; folic acid, 2; inositol, 2000; niacin, 100; Ca-pantothenate, 150; pyridoxine-HCl, 30; riboflavin, 20; and thiamine-HCl, 20.
- 3) Sorbic acid and propionic acid.

that these essential amino acids, or at least some of them, are not synthesized at all by the silkworm. It is reported that the radiocarbon of labelled glucose is incorporated, though slightly, into these amino acids,³⁹⁾ and that they are slightly involved in transamination in larval tissues.^{9),35)} The lack of the supply of any essential amino acid may result in a cessation of the protein synthesis in the animal.

There is additional evidence for the occurrence of pathways involved in the synthesis of the essential amino acids in the silkworm. Radiochemical experiments have demonstrated that arginine is formed from citrulline.^{15),20)} However, since ornithine is not actively converted to citrulline by the larva, arginine formation must be insignificant. The silkworm is able to convert slowly phenylpyruvate to phenylalanine,¹³⁾ and indole lactic acid to tryptophan,^{13),32)} but it is also wholly unlikely that these precursors occur at a substantial amount in the silkworm.

2. Essential amino acids (group-2). *Aspartic and glutamic acids.*

Single omission of these acidic amino acids from a diet affected larval growth slightly, but double omission resulted in a marked suppression of growth.²⁴⁾ It was subsequently demonstrated that the silkworm requires either of them as an essential amino acid.²⁶⁾ Their nutritive value was nearly equivalent each other. It must still be clarified whether both of them are needed or either one is sufficient for supporting normal growth.

Aspartic and glutamic acids are interconverted by a transaminase in larval tissues.^{3),34)} They are also involved in transamination for synthesis of alanine and glycine, the main constituents of silk protein. Aspartic or glutamic acid can be replaced only slightly by a large amount of dietary alanine, glycine, or serine.²⁷⁾

Glutamic acid is readily oxidized by larval tissues.²⁹⁾ It is considered that it is converted to α -ketoglutaric acid by glutamic acid dehydrogenase, linked to the tricarboxylic acid cycle.

3. Semi-essential amino acid. *Proline.*

It was at first reported that proline is essential for the silkworm,^{1),24)} because larval growth was retarded markedly by its absence. A subsequent deletion experiment using an improved diet showed that it is semi-essential,²⁾ because even in its absence the larva was able to grow, though slowly, and reached the next instar. However, the weight increase was still inferior to the control, and a smaller cocoon was obtained when the fifth-instar larva was reared throughout on the proline-deficient diet.

Proline is synthesized from its precursors via the ornithine cycle in the silkworm.^{15),20)} The proline requirement was spared partly by glutamic acid, arginine, or ornithine, but an excess of arginine in place of proline was detrimental. The requirement for proline as a semi-essential amino acid suggests that proline is synthesized at inadequately low level in the silkworm.

4. Non-essential amino acids (group-1). *Alanine, glycine, and serine.*

Deleted singly, these amino acids scarcely affected larval growth and were considered to be non-essential.^{1),24)} The addition of each of them to the diet lacking both aspartic and glutamic acids improved the dietary efficiency to some degree, however, the improvement was inferior to that found with the acidic amino acids.²⁷⁾ Furthermore, the addition of all three to diet containing acidic amino acids somewhat improved larval growth and silk production.

As mentioned above, these amino acids are readily synthesized by transamination. Other metabolic pathways are also available for

their synthesis. Furthermore, interconversion of alanine, glycine, and serine are reported for the silkworm.^{4),7),9)-11),34),35),37)} Substantial amounts of alanine, glycine, and serine, which are utilized in the synthesis of larval tissues or cocoon silk during the fifth instar, have been shown to be derived with from *de novo* synthesis.^{12),33),35)}

5. Non-essential amino acids (group-2). *Cystine and tyrosine.*

Single omission of cystine and tyrosine scarcely retarded larval growth, and they were concluded to be dispensable.^{1),24)} However, their nutritive value cannot be ignored, because it is closely related to the dietary levels of methionine and phenylalanine, respectively.²⁷⁾ In diet containing little or no methionine or phenylalanine, added cystine or tyrosine had no improving effect. In the presence of insufficient methionine and phenylalanine, however, cystine and tyrosine were able to replace methionine and phenylalanine, respectively, acting as if they were indispensable. A considerable amount of methionine and phenylalanine supplied from the diet may have to be metabolized to cystine and tyrosine by the larva. Cystine and tyrosine still produced a slight growth-promoting effect even in the presence of sufficient methionine and phenylalanine. Conversion from methionine to cystine via cystathionine¹⁴⁾ and from phenylalanine to tyrosine^{5),8)} has been demonstrated in the silkworm by radiochemical studies.

A classification of L-amino acids on the basis of their nutritional and metabolic roles in the silkworm has been represented here. It seems necessary to add a few remarks on the amino acid nutrition in the silkworm. The occurrence of substantial amounts of the free cystathionine^{14),18),21),36),38)} and lanthionine,^{18),21),38)} which are not found in mulberry leaves, has been observed in hemolymph. It is interesting that the female is characterized by a high concentration of these sulfur amino acids.^{17),18),21)} Their concentrations depend on the levels of dietary methionine and cystine.¹⁸⁾ The analysis of quantitative requirements for amino acids and of balances between dispensable and indispensable amino acids provides information for improvement not only of larval growth but also of cocoon yield.^{13),28)} Data in this field have been accumulating. Further, dietary conditions, such as the omission or change in level of a specific amino acid, affect the amino acid pattern of hemolymph¹⁶⁾ and the rate of conversion of amino acids. Such a nutritional control involves metabolic adaptation. A review on this subject has been published recently.²³⁾

Nutritive effects of D-amino acids were tested by including them singly in place of L-isomers and by examining the alteration of free amino acid pattern of hemolymph of larvae fed on the D-amino acid diet.³¹⁾ Effects of D-isomers on the silkworm differ according to the

various D-isomers. (i) D-Amino acids which possess a slight nutritive effect are D-histidine and D-methionine. (ii) D-Leucine, D-alanine, and D-serine are toxic. There seem to be still a few other toxic D-amino acids. (iii) Many of the D-isomers are considered to be neither toxic nor of nutritive effect.

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References

- 1) Arai, N., and Ito, T.: J. Seric. Sci. Japan, **33**, 107 (1964).
- 2) —: Bull. Seric. Exp. Sta., **21**, 373 (1967).
- 3) Bheemeswar, B., and Sreenivasaya, M.: Curr. Sci., **21**, 253 (1952).
- 4) Bricteux-Grégoire, S., Dewandre, A., Florkin, M., and Verly, W. G.: Arch. Int. Physiol. Biochim., **67**, 693 (1959).
- 5) Bricteux-Grégoire, S., Verly, W. G., and Florkin, M.: Nature, **177**, 1237 (1956).
- 6) Dougherty, E. C.: Ann. N. Y. Acad. Sci., **77**, 27 (1959).
- 7) Florkin, M.: Proc. 4th Int. Congr. Biochem. Vol. 12 (ed. by Levenbook, L.), pp. 63–77. Pergamon Press, London (1959).
- 8) Fukuda, T.: Nature, **177**, 429 (1956).
- 9) —: J. Biochem., **44**, 505 (1957).
- 10) —: ibid., **45**, 469 (1958).
- 11) —: ibid., **47**, 720 (1960).
- 12) Fukuda, T., Duchâteau-Bosson, G., and Florkin, M.: Arch. Int. Physiol. Biochim., **69**, 701 (1961).
- 13) Horie, Y., Inokuchi, T., and Watanabe, K.: Bull. Seric. Exp. Sta., **24**, 345 (1970).
- 14) Inokuchi, T.: ibid., **23**, 371 (1969).
- 15) —: ibid., **23**, 389 (1969).
- 16) —: ibid., **24**, 389 (1970).
- 17) —: J. Seric. Sci. Japan, **41**, 44 (1972).
- 18) —: Bull. Seric. Exp. Sta. (in press).
- 19) Inokuchi, T., Horie, Y., and Ito, T.: ibid., **22**, 195 (1967).
- 20) —: Bioch. Bioph. Res. Comm., **35**, 783 (1969).
- 21) Inokuchi, T., and Ito, T.: J. Seric. Sci. Japan (in press).
- 22) Ito, T.: Proc. Japan Acad., **43**, 57 (1967).
- 23) —: Israel J. Entomol. (in press).
- 24) Ito, T., and Arai, N.: Bull. Seric. Exp. Sta., **19**, 345 (1965).
- 25) —: J. Agric. Chem. Soc. Japan, **40**, 110 (1966).
- 26) —: J. Insect Physiol., **12**, 861 (1966).
- 27) —: ibid., **13**, 1813 (1967).
- 28) Ito, T., Arai, N., and Inokuchi, T.: Bull. Seric. Exp. Sta., **21**, 385 (1967).
- 29) Ito, T., Horie, Y., and Ishikawa, S.: J. Insect Physiol., **2**, 313 (1958).
- 30) Ito, T., and Inokuchi, T.: Addressed to the Conference of 'Significance of Insect and Mite Nutrition', Lexington, Kentucky, April (1972).
- 31) —: in preparation.
- 32) Kikkawa, H.: Science, **111**, 495 (1950).
- 33) Kirimura, J.: Bull. Seric. Exp. Sta., **17**, 447 (1962).
- 34) Koide, F., Nagayama, H., and Shimura, K.: J. Agric. Chem. Soc. Japan, **29**, 987 (1955).

- 35) Koide, F., Shishido, T., Nagayama, H., and Shimura, K.: *ibid.*, **30**, 283 (1956).
- 36) Kondo, Y.: *J. Biochem.*, **51**, 188 (1962).
- 37) Nagayama, H., and Shimura, K.: *ibid.*, **49**, 55 (1961).
- 38) Rao, D. R., Ennor, A. H., and Thorpe, B.: *Biochemistry*, **6**, 1208 (1967).
- 39) Shyamala, M. B., and Bhat, J. V.: *Indian J. Biochem.*, **2**, 201 (1965).