Hormonal Control of Silk Production in Silkworm, *Bombyx mori*

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The domesticated silkworm, *Bombyx mori* L., is one of the most useful insects in agriculture. Before the World War II, Japanese sericulture was the most important export industry, and produced about 400,000 ton of the silkworm cocoon. Although it was decreased extremely during the War, this industry is still important in Japanese agriculture.

Bombyx mori is classified as belonging to the order Lepidoptera, the suborder Heteroneura, and the family Bombycidae (Tazima et al., 1975). Newly hatched larvae grow very quickly, feeding solely on mulberry leaves, and they become mature larvae after 20–23 days, then spin cocoon with silk materials from fully-grown silk gland. The silk gland produces a large amounts of various silk proteins (fibroin and three kinds of sericins). The gland is a large, tubular gland, and is composed with anterior, middle, and posterior divisions (Fig. 1). The main fibrous component of silk fibroin is produced in the posterior division of the gland, while the gelatinous component, sericin, which coats the fibroin, is secreted in the middle division.

The silk gland is also one of the most effective organ for insect hormones, especially, ecdysone and juvenile hormone (JH). Growth and differentiations of the gland cells are accelerated by ecdysone, and are controled by JH. JH administrations induced additional accumulation of silk protein in the gland (Akai et al., 1971). Methoprane, a kind of synthetic JH analogues, was developed as a commercial drug in 1977, and named "Manta". The Manta is starting to be used by Japanese sericultural farmers to produce more silk.

In this paper, the author will describe the recent studies and the progress on hormonal control of silk production in silkworm, especially, structure and function of the silk gland, hormonal control of silk-protein synthesis, and utilization of JH for sericulture.

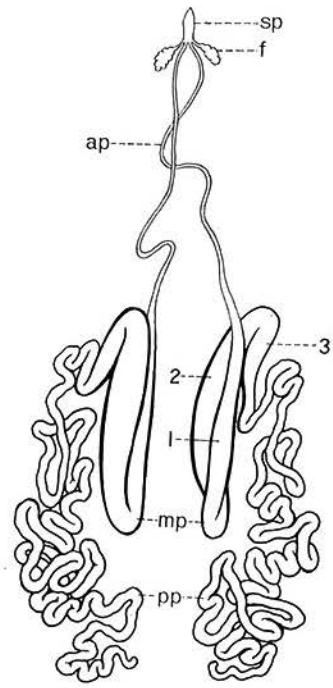

Fig. 1. Schematic drawing of the silkgland of *Bombyx* larva at the mature stage. ap: anterior division, mp: middle division, 1: anterior piece of middle division, 2: middle piece, 3: posterior piece, pp: posterior division, sp: spinneret, f: Filippi's gland

Structure and function of silk gland

Since the author studied ultrastructure of the posterior silk gland which produces only fibroin (Akai, 1963; 1964; 1965), many information concerning ultrastructure and function have been accumulated (Akai and Kobayashi, 1966; Tashiro et al., 1968; Matsuura et al., 1968; Akai, 1971; Iijima, 1971a; 1971 b). Characteristic ultrastructural changes of the gland cells during larval development were also reviewed (Akai, 1976).

The nucleus contains numerous nucleoli and chromatin bodies, and they ramified extremely like a mesh-work with the lapse of time of larval development. Also, the nucleoli develop

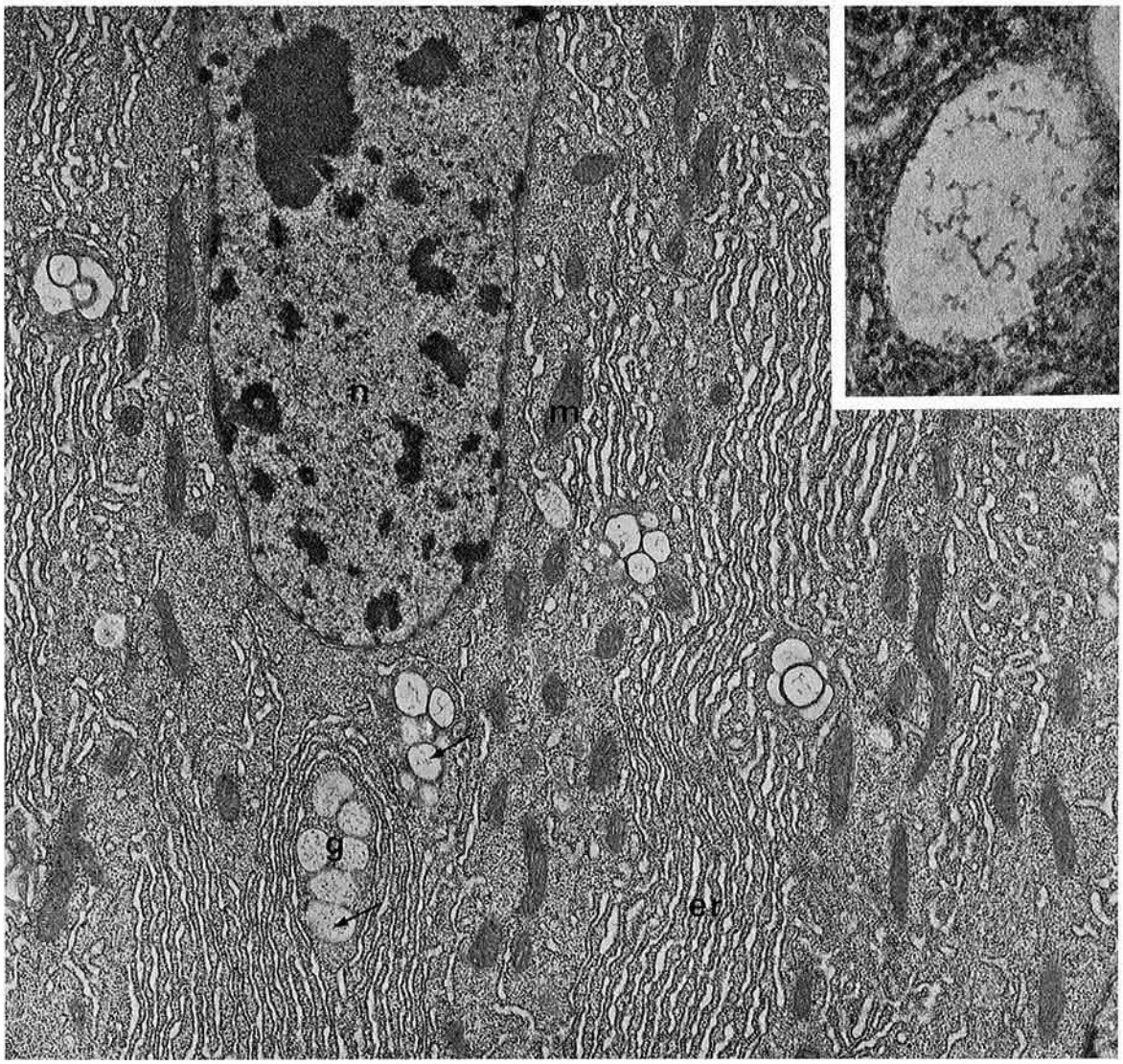

Plate 1. Electron micrograph of posterior silkgland at the mature stage of *Bombyx* silkworm. Fibroins (arrows) are concentrated in the Golgi complexes. er: granular endoplasmic reticulum, g: Golgi complexes, m: mitochondria, n: nucleus. ×15,000.
Inset: Filamentous fibroins are seen in a Golgi vacuole. ×70,000

enormously during the most active period of RNA synthesis, and incorporate labelled uridine into them in same times.

In the cytoplasm, granular endoplasmic reticulum, Golgi complexes and fibroin globules are also conspicuous during the duration of active fibroin synthesis. Labelled glycine which is one of the main composing amino acids of the fibroin, is incorporated into the granular endoplasmic reticulum at first, then, transfered into the Golgi complexes, fibroin globules, and finally, into the liquid fibroin in the lumen (Akai and Kobayashi, 1965). In both Golgi vacuoles and fibroin globules, elementary fibers of fibroin which presumably represent newly synthesized fibroin molecules transported from the granular endoplasmic reticulum, were detected (Akai, 1971). Each elementary fiber in the Golgi vacuoles was seen as a helical bundle of five to seven threads. The bundles were about 130 Å in diameter, and the threads were 20 to 30 Å in diameter (Plate 1.). These elementary fibers are only a fundamental structure in the liquid fibroin in the gland. The major liquid fibroin in the lumen is consisted with numerous spherical masses of the elementary fibers (Akai and Kataoka, 1978).

Hormonal control of silk production in silk gland

As mentioned above, the silk gland is one of the most responsive organs to insect hormones, ecdysone and JH. The former is secreted from prothoracic glands, and the latter from corpora allata. When the corpora allata were removed from early 4th instar larvae, they become precocious pupae without the 4th larval molting. During this process the silk gland shows hypertrophic development like a developmental pattern of the 5th instar (Fukuda, 1942; Akai and Kiguchi, 1978). By the electron microscopic observation, enormous amounts of fibrous fiber are secreted from the posterior silk gland of the allatectomized larvae as compared with normal

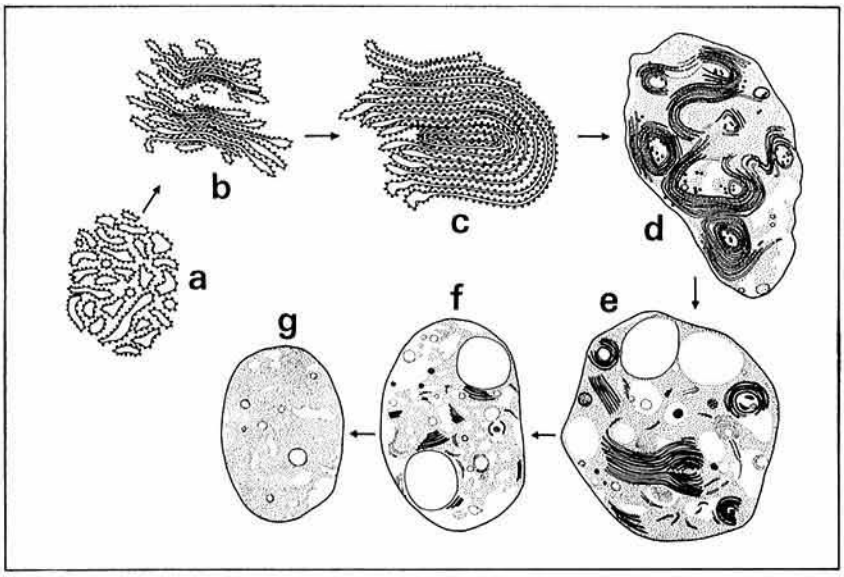

Fig. 2. Ultrastructural changes of cytoplasmic organelles in the posterior silkgland cell by an administration of ecdysterone. (a): Granular endoplasmic reticulum (er) just after the ecdysterone administration, (b): Lamellar-like er after 6–12 hrs of the administration, (c): Lamellar structure of er after 12–18 hrs, (d): Autophagosome enclosed by a membrane after 12–24 hrs, (e)—(g): Progressed lysosome after 24–48 hrs.

Table 1. Influence of JH on ultrastructure of intracellular-transport system of fibroin in posterior silkgland cell of *Bombyx* larvae

Doses of administered JH (μg/animal)	Diameter of Golgi vacuoles (μm)	Number of fibroin element in Golgi vacuoles	Diameter of fibroin globules (μm)	Number of fibroin element in fibroin globules
30	0.28	6.1	0.35	28
0.3	0.38	21.8	0.45	about 65
0 (control)	0.43	45.7	0.53	about 85

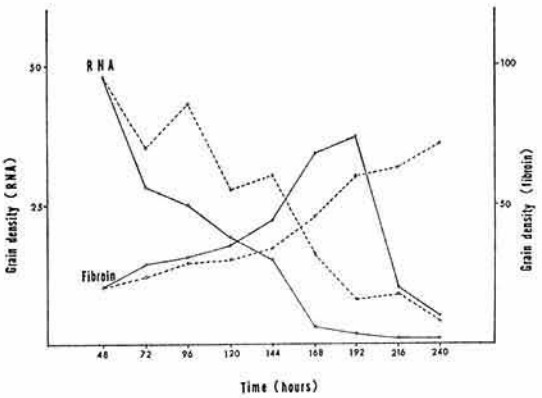

Fig. 3. Effects of JH on RNA and fibroin synthsis in the posterior silkgland of *Bombyx* larvae during the 5th instar. Solid line: controls. Dotted line: JH-injected. The data for both RNA and fibroin were based on grain counts (grains per $20\mu^2$) on nuclei and cytoplasm, respectively.

4th instar larvae (Akai and Kiguchi, 1978).

By the administration of considerable amounts of ecdysone in middle stage of the 5th instar, the silk gland cells are rapidly changed toward the histolysis producing numerous lysosomes. In Fig. 2, the process of the formation of autophagosome induced by ecdysone administration is shown. At several hours after the ecdysone administration, the granular endoplasmic reticula are concentrated in everywhere, and then, they are enclosed by a separate membrane with some mitochondria and ground matrix. Finally, they become lytic vacuoles through the autophagosome. Similar ultrastructural changes in the gland cell occur during larval and pupal molting stages. During the larval-pupal metamorphosis, however, the silk gland cells are

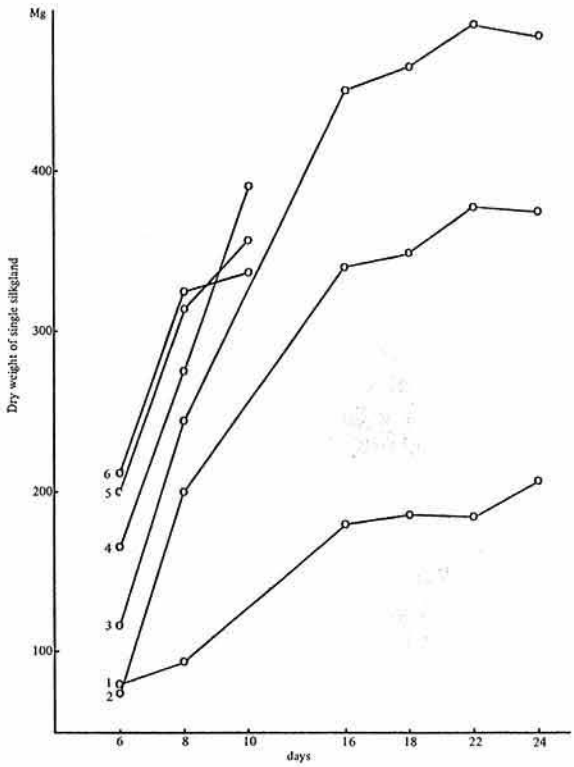

Fig. 4. Influence of JH on dry weight of silkgland of *Bombyx* larvae
Different doses of JH were administered on the 1st day of the 5th instar. 1–6: doses of administered JH (30, 3, 0.3, 0.03 μg/animal, and control).

histolysised completely.

On the other hand, JH administration controls the larval period and silk production by the supplied amounts and developmental stages. In the early experiments, we found that 10 μg JH administration prevented any pupal development including spining for co-

Table 2. Effects of Manta (JH) on cocoon quality of *Bombyx mori* reared in spring rearing season

Dose of Manta	Sex	Cocoon weight (g)	Average	Cocoon-shell weight (cg)	Average	Pupa weight (g)	Average
500 times diluted solution	female	3.78		72.2		3.04	
	male	2.86	3.32 (115)	68.4	70.3 (112)	2.12	2.58 (117)
100 times	female	4.13		78.3		3.32	
	male	2.95	3.54 (122)	70.4	74.4 (119)	2.23	2.78 (126)
50 times	female	4.17		77.1		3.37	
	male	2.99	3.58 (124)	71.3	74.2 (118)	2.26	2.82 (128)
Control	female	3.40		66.0		2.64	
	male	2.38	2.89 (100)	59.4	62.7 (100)	1.77	2.21 (100)

(Kobari and Akai, 1978)

coon formation. Such larvae were called as "dauer larvae". Their silk glands contained a large amount of liquid silk in the lumen, and the gland cells were well developed and showed no signs of histolysis (Akai and Kobayashi, 1971).

In next experiments, we found that higher doses of JH at the former half of the 5th instar (0–72nd hour after 4th ecdysis) extended the feeding for an extra day. Both the cocoon weight and the cocoon shell weight of these animals were increased. These larvae produced additional about 30% more silk than the controls. In this case, RNA synthesis in the silk gland showed considerable differences between the test and control animals as shown in Fig. 3. The synthetic activities of JH-injected 5th-instar-larvae were higher than those of the control animals. The protein synthesis in the gland of JH test-animals was slightly lower until the 192nd hour after the 4th ecdysis. However, the activity of the control animals decreased rapidly after the 192nd hour of the 5th instar. In JH test-animals, the synthetic activity continued for another one or two days. Therefore, there was a substantial net synthesis of fibroin induced by JH.

Recently, the author studied the influences of graded doses of JH administration on the intracellular-transport system of fibroin in the posterior silk gland cell by means of electron microscope. As shown in Table 1, higher dose of JH controls the development of these system, such as, granular endoplasmic reticulum, Golgi complexes, fibroin globules, and the number of fibroin elements in these organelles (Akai, 1978). However, the fibroin elements in these system increased by the administration of suitable amounts of JH. Dry weight of the silk gland was also controlled by JH amounts as shown in Fig. 4.

Utilization of JH for silk production in sericulture

Since the studies on the influences of JH on the growth and metamorphosis of *Bombyx* larvae were published by Akai et al. (Akai and Kobayashi, 1971; Akai et al., 1971; 1973), and the fact that the prolongation of feeding period and increase of both cocoon and cocoon shell weight are induced by JH administrations was made clear, many workers confirmed the fact by using several synthetic JHs

(Chang et al., 1972; Nihmura et al., 1972; Murakoshi et al., 1972; Muroga et al., 1975; Aomori et al., 1977). As mentioned before, Manta was developed as a commercial drug for increasing silk in sericulture in 1977. In order to confirm the efficiency of the Manta for the silkworm rearing, the practical administration tests were carried out for the silkworms on mulberry leaves (Kobari and Akai, 1978). By the administration of the standard Manta solution during the 48th to 60th hour of the 5th instar, cocoon shell weight increased about 10% constantly through three rearing seasons. In the case of the administration of large amounts of standard Manta solution, any problems were not detected in the development of the silkworms including non-spinning individuals and cocoon quality. Highest increase of cocoon production by the Manta administration was constantly shown by the treatment at the 72nd hour of the 5th instar. In the cases of the administration of higher doses of Manta solutions in autumn rearing season, cocoon quality was more increased in both cocoon shell weight (10–18%) and pupal weights. In most recent tests of Manta, we obtained very big cocoon, including 940 mg of single cocoon shell weight, and about 20% increase in average index than that of the controls (Table 2) (Kobari and Akai, in preparation).

From these results, the author emphasizes the need of more detailed study concerning the most effective administration times and doses for different nutritional factors, rearing environments, and silkworm races. When this technique is more improved and popularized all over the country, it will undoubtedly be useful to increase the cocoon yield in Japanese sericulture.

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