Hormonal Regulation of Larval Development and Its Utilization in Silk Production by *Bombyx* Silkmoth

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Introduction

When silkworm larvae were ligated with a fine silk thread at a point between their thorax and abdomen, pupal characters were induced in the head and thorax, but not in the abdomen. In another case, when the corpora allata were extirpated from early 3rd instar larvae, they became pupae directly without any larval molt. This type of endocrinological experiment started early in this century, and has revealed that the silkworm development is controlled by insect hormones, as is true in other animals.

Concerning the insect endocrine organ, such as brain, subesophageal ganglion (SG), corpora cardiaca (CC), corpora allata (CA), and prothoracic gland (PG), they have been found to play a part in the developmental regulation of the life cycle (Fig. 1). In the brain several types of neurosecretory cells distribute and secrete neurohormones: prothoracicotropic hormone (PTTH), allatotropic hormone, eclosion hormone (EH), melanization and reddish hormone (MRCH), and bursicon. PTTHs were isolated and their chemical structures revealed[13]. SG secretes diapause hormones (DH) from its neurosecretory cells and CC secretes an adipokinetic hormone (AKH). CA, which is a common endocrine organ in insects, secretes a juvenile hormone (JH). To date, four kinds of JH (JHI, JHII, JHIII, and JHO) have been found, their chemical structures determined, and numerous JH analogues which are accompanied by the biological activities have been synthesized. PG secretes a molting hormone called ecdysone. In post-embryonic development of the insect, both ecdysone and JH are essential, especially in larval molt and metamorphosis. Both hormones are controlled

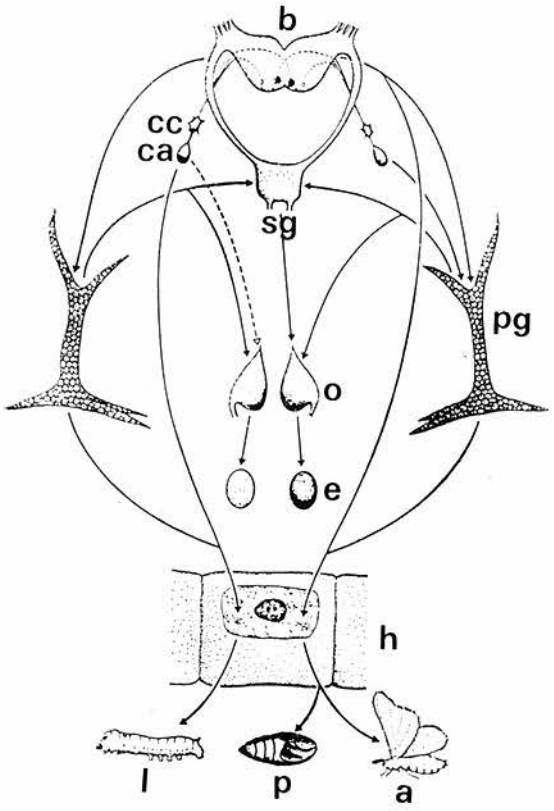

Fig. 1. Endocrine system of silkworm
a: adult, b: brain, ca: corpora allata, cc: corpora cardiaca, e: egg, h: hypodermis, l: larva, o: ovary, p: pupa, pg: prothoracic gland, sg: subesophageal ganglion.

by the neurohormones from neurosecretory cells in the brain.

In this review the author briefly reviews the hormonal regulations of insect development including larval molting, larval-pupal metamorphosis, and their utility in the silk production of *Bombyx mori* as found from the work in his laboratory.

Brain-corpora cardiaca-corpora allata system

When the insect brain is stimulated by a light factor, the neurohormones stored in neurosecretory cells located in the brain are released into the hemolymph via their axons, terminating in the CC or CA. Release of PTTH stimulates ecdysone secretion from PG cells. Meanwhile, allatotropin which stimulates JH secretion from the CA is transferred from neurosecretory cells in the brain into CA.

By light and electron microscopic observations, it was confirmed that the neurosecretory cells distribute in median and lateral parts of the brain in clusters. These neurosecretory cells contain numerous neurosecretory granules ranging 100–200 nm in diameter. In *Bombyx* larvae, large characteristic A cells, each containing a large vacuole are found[12], and the electron microscope revealed that the vacuole to be filled with fine fibrous materials[3]. Although, as mentioned, neurohormones are known to be secreted from neurosecretory cells in the brain, it is not yet clear what cell secretes which kind of hormone. The special cells which secrete PTTH in median and lateral neurosecretory clusters, however, have been identified[1]. In the lateral neurosecretory clusters in *Bombyx* larvae, typical neurosecretory cells containing neurosecretory granules are found. In these cells the granules are formed by the rough endoplasmic reticulum—Golgi complex system in the cytoplasm, and transferred into the CC nerve via axons. By the electron microscope the nerve is shown to contain 30–40 axons with four types of neurosecretory granules and several axons without any granules.

The CC shows a complicated morphology, the organ is organized with several neurosecretory cells, numerous neurosecretory axons containing opaque granules, glial cells, and a tracheal system[2]. The CA is visible as a spherical organ containing many secretory cells (estimated at 20–30). The neurosecretory axons containing opaque granules and empty capsules from brain penetrate into the

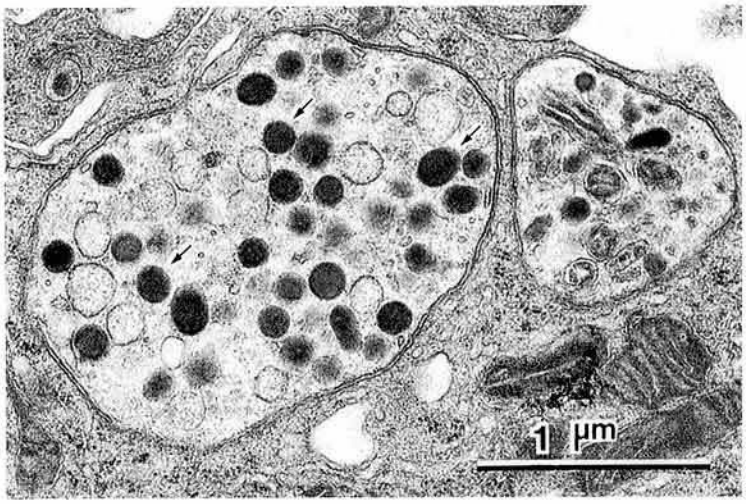

Plate 1. Electron micrograph of neurosecretory axons penetrating into corpora allata
Arrows indicate neurosecretory granules.

CA cells (Plate 1). At least three types of neurosecretory granules are detected in the axons, a large size (150–250 nm in diameter), medium (100–150 nm), and small (50–75 nm)[2], but the cells from which they originate are not yet elucidated.

Bombyx larvae display a well developed smooth endoplasmic reticulum and Golgi complexes in CA cells which show a periodical secretory activity.

Prothoracic gland and ecdysone secretion

PGs have no neurosecretory axon from the brain, and they receive the stimulation of PTTH via the hemolymph. In *Galleria* larvae, several PG cells remaining after extirpation of the PG become very much larger, showing their compensational function[14]. In *Bombyx mori*, ecdysteroid titer greatly increases (about 1500 ng/ml hemolymph) during the spinning period in the final instar. During this period, characteristic ultrastructural changes occur in PG cells: mitochondrial changes and lysosome and slit-like vacuole formations in the cytoplasm. At the beginning of the spinning period, numerous slit-like vacuoles appear in the cytoplasmic area, disappearing 1–2 days later. Anti-juvenoid (imidazol compound, KK-42) treatment inhibits ecdysteroid titer in hemolymph in *Bombyx* larvae as shown in Fig. 2. By comparative ultrastructural observations of PG between normal and KK-42 treated larvae, the slit-like vacuoles seem to be concerned with the ecdysone pool in the cell and with the change of ecdysteroid titers. In KK-42 treated larvae, the slit-like vacuoles remain 4–5 days and then decrease gradually according to the increase of ecdysteroid titer[5].

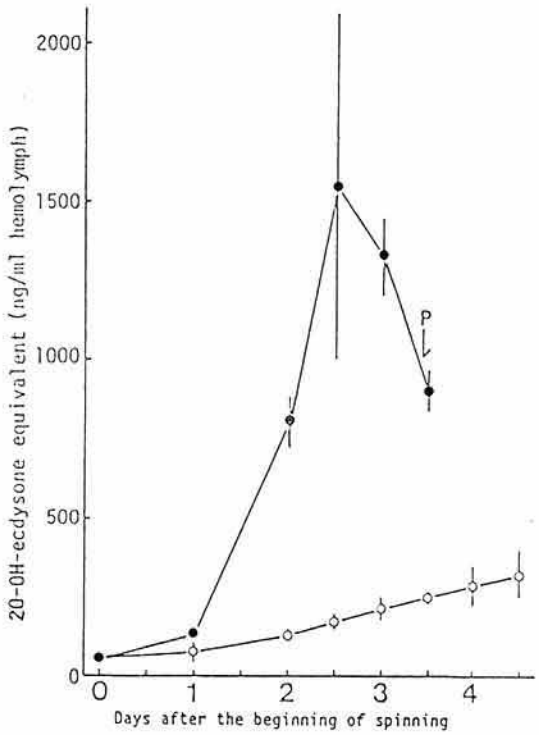

Fig. 2. Changes of ecdysteroid titers in hemolymph after the treatment of KK-42
• : Control,
○ : KK-42-treatment,
P: Pupation.

Control of growth by insect hormones

The growth of silkworms, especially those at the larval stage, is controlled by the endocrine system, which is mainly ecdysone and JH. Ecdysone secreted from the prothoracic gland and JH produced by CA are most directly concerned with silkworm growth. While the former induces molting, the latter helps retain juvenile characters in their life cycle. When the former exists in the hemolymph at high concentration, pupation is induced, and when the latter works in a high concentration of ecdysone, larval molting takes place.

The chemical structure of ecdysone and JH has been clarified, and a number of compound analogues to these two hormones have been extracted from nature and synthesized artificially. By administering these substances which activate insect hormones to silkworms and thus changing the hormone titers in the insect hemolymph, it is possible to extend the larval period and control the frequency of larval molt. Our laboratory succeeded in controlling the growth of silkworm larvae and, as a result, in controlling the size of cocoons

and thickness of cocoon filaments.

Control of growth and cocoon production by JH analogues

In 1967, Dr. H. Roller, professor at the University of Wisconsin in the United States, succeeded in isolating JH. When the JH was administered to silkworms topically, the larval period was greatly extended[6]. Thereafter, various synthesized JH analogues were used to study growth control by JH and to determine the influence of JH on the silk protein synthesis.

When a large quantity of JH is given to silkworms in their 5th instar, their metamorphosis to pupate is controlled, and they remain longer as larvae, becoming so-called "dauer larvae". If the treatment of JH is reduced, the extension of the larval period stops within one to three days and the insects can spin and pupate. In this case, cocoon shell weight increases by 10 to 40%[4].

Examination of the timing of JH treatment to silkworms from a viewpoint of improving cocoon productivity revealed that when JH was administered around the 3rd day of the 5th instar, the increase of cocoon shell weight was greatest, showing the great practical potential of this technique. In 1977, JH began to be sold on the market under the commercial name of "Manta". Spraying a diluted solution of Manta over a silkworm rearing bed once during the 5th instar results in an increase in cocoon production of more than 10%. Sericultural farmers are using Manta to raise their cocoon yield and filament production.

The relation between JH treatment and the synthesis of silk protein in the silk gland of larvae has also been clarified. When an appropriate quantity of JH is treated to larvae in the early stage of their 5th instar, the activity of RNA synthesis in the cells of the glands lasts longer than in the control group, resulting in a longer period of silk protein synthesis. Thus, it is clear that the total volume of silk protein synthesized is increased by JH topical treatment[4].

Later, on a laboratory test basis, it was found that cocoon production could be raised by over 20% by successive treatments (once during the 3rd, 4th and 5th instars) of Manta rather than only once during the 5th instar[9].

The relation between the effect of greater cocoon production by JH treatment and the genetic characters of silkworms was also investigated. In general, the effect of JH treatment was higher in hybridized strains than in the original strain, and among hybrids the effect was greater in healthy ones. The treatment had a greater effect in the healthiest strains which produced a great deal of filament, such as HN90×HC74 (renamed NO2×CO2). In Japan, the average cocoon shell weight is 55 to 58 cg in the spring rearing season when the rearing conditions are the best. Japanese silkworm breeders had once believed that it would be almost impossible to turn out cocoons of over 1 g, but as a result of JH treatment, cocoons weighing 1.19 g by female silkworms and 1.12 g by male are obtained[7].

Control of growth and cocoon production by anti-JH

The substance that works against JH was extracted from plants and is called "anti-juvenile hormone (anti-JH)"[10]. We used two types of imidazole compound, which has such anti-JH effects as induction of tetramolters into trimolters and causing smaller cocoon filaments.

Ordinary silkworms are tetramolters and make cocoons in their 5th instar. The silkworms called trimolters make cocoons in their 4th instar, therefore, trimolters are smaller than tetramolters. As the maximum thickness of cocoon filaments is largely controlled by the size of the orifice of the spinneret of a silkworm, the size of the filament produced by trimolters is far smaller than that of the filaments made by tetramolters. By giving anti-JH orally to silkworms in each of their instars, we succeeded in changing the number of larval molts and in changing greatly the length of the larval period, cocoon size and size of cocoon filament[8]. Fig. 3 shows the larval

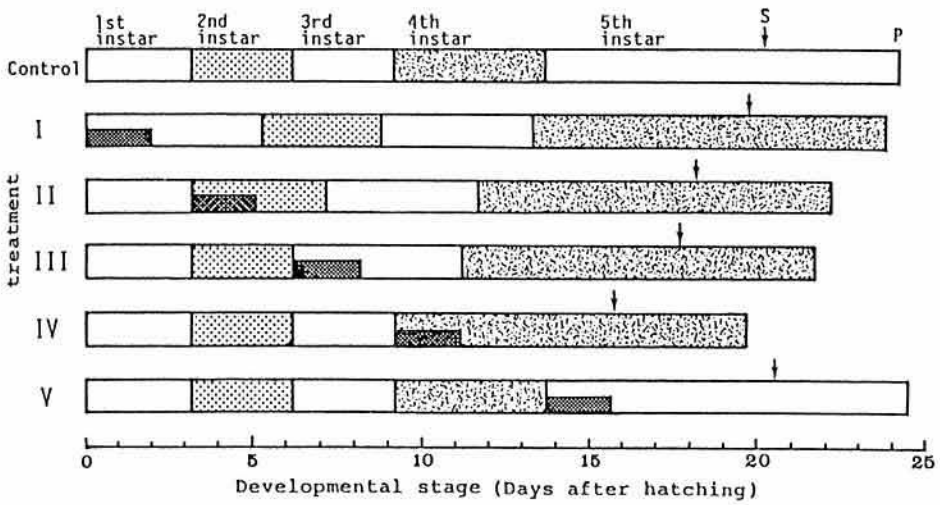

Fig. 3. Larval development of anti-JH treated larvae

Control: Length of each instar and spinning time (indicated by S) of the control (tetramolters).

I : Larvae treated with anti-JH in their 1st instar and turned into trimolters. ▩ shows the time of anti-JH administration.

II : Larvae treated with anti-JH in their 2nd instar and turned into trimolters.

III : Larvae treated with anti-JH in their 3rd instar and turned into trimolters.

IV : Larvae treated with anti-JH in their 4th instar and turned into trimolters.

V : Larvae treated with anti-JH in their 5th instar.

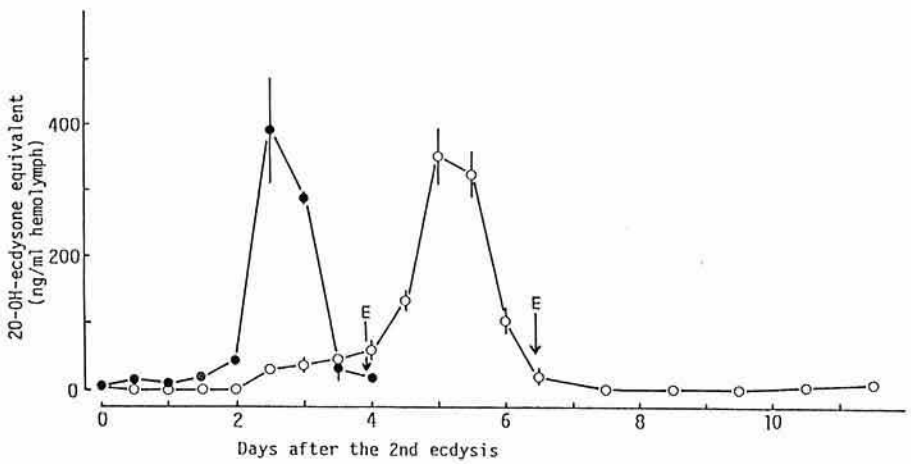

Fig. 4. Hemolymph ecdysteroid level of the control and KK-42-treated 3rd instar larvae

● : Control,

○ : Dietary administration of KK-42 for 48 hr from the commencement of feeding at the 3rd instar.

E : Ecdysis.

development of silkworms to which anti-JH was administered. Common tetramolters spend about 3 days for each of the 1st and 2nd instars, 5 days for their 4th instar and 10 days for their 5th instar. When anti-JH is given to them in their 1st instar, the 1st instar is extended by 2 days or so, and about 60% of the tetramolters are induced into trimolters. When anti-JH administration is made in their 2nd instar, the 2nd instar becomes longer and some 90% are converted into trimolters. In the case of anti-JH administration in the 3rd instar, the 3rd instar is extended and the conversion ratio is 100%. When anti-JH is given in the 4th instar, the 4th instar is extended to a similar length as the 5th instar at about 100% ratio[11]. Anti-JH administration in the 3rd and 4th instars results in about 100% conversion of tetramolters into trimolters and so is the most promising and practical technique. Usually, the cocoon filament size shows a proportional response, and anti-JH treatments can produce different size of cocoon filament.

Elucidation of the mode of action of anti-JH is an important matter, so we studied the ecdysteroid titers of normal development and anti-JH treated trimolter larvae by radioimmunoassay. Fig. 4 shows the ecdysteroid titers of both normal and anti-JH treated larvae. By anti-JH treatment in the early period of 3rd instar the peak of ecdysteroid titer was delayed about 2.5 days, with the result that ecdysis was delayed.

During the 1st to 4th instar the ecdysteroid peaks are near 400 ng/m*l* hemolymph in every instar, but the titers of final larval instars including the 4th instar of trimolters greatly increase at the level of 1500 ng/m*l* hemolymph, showing the necessity of a higher level of ecdysteroid titer in larval-pupal metamorphosis than in the larval molt.

On the other hand, when the corpora allata was extirpated at 12 hours of the 4th instar, pupal molt took 7 days later and the ecdysteroid titer showed the characteristic pattern

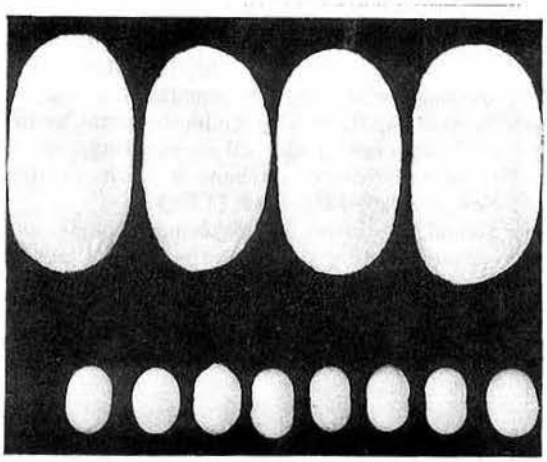

Plate 2. Comparison of two kinds of cocoons, from pentamolters induced by ecdysone (upper) and dimolters by anti-JH treatment

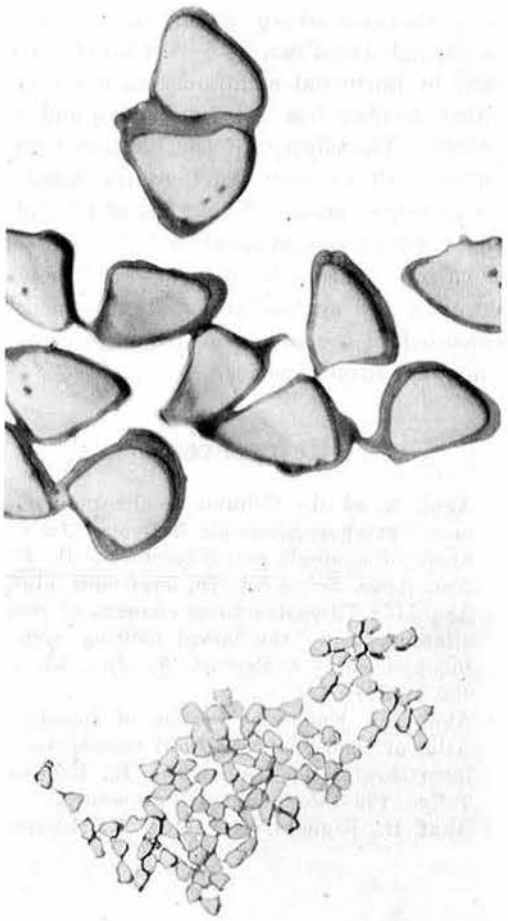

Plate 3. Comparison of two kinds of cocoon filaments, from the pentamolter cocoons and the dimolters in Plate 2

of the last larval instar. Thus, the pattern of ecdysteroid titer changes from the larval to the pupal molt.

In an other case, when an artificial diet containing a lower dose of ecdysterone was given to larvae throughout an entire instar, the larvae became the 6th instar and then giant larvae later. When the 4th instar larvae were given a moderate dose of ecdysterone, the 4th and 5th molts took place and produced large cocoons accompanied by large size cocoon filaments (Plates 2, 3). In contrast, an artificial diet containing a moderate dose of anti-JH can induce dimolter larvae which spin an extremely small cocoon with very fine cocoon filament (Plates, 2, 3).

From these results, it was demonstrated that a silkworm strain having genes identical to a normal strain can be controlled to a large extent by hormonal manipulation, not only in molting number but also insect size and productivity. The efficacy of the hormonal manipulation will be even further increased by greater improvement. This type of manipulation of insect development will be utilizable for various insects in future in addition to silkworms and agricultural pests, because the mechanism of the endocrine system is common throughout insect species.

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