80. *Notes on the Growth of Cuticle in the Silkworm.*

By Zyuiti KUWANA.

Imperial Sericultural Experiment Station, Tokyo.

(Comm. by C. SASAKI, M.I.A., June 12, 1933.)

In order to determine whether the production of cuticular substances from the hypodermal cell takes place continuously or periodically, experiments were carried out with a Japanese race (No. 107.) of silkworms. The results obtained are given in the descriptions that follow.

Thickening of cuticle. On examination, the material was fixed with Carnoy's and Champy's fluid and 10% formol, and the thickness of the cuticle was measured in the cross section of the thoracic segment of the fifth instar. So far as observations go, the cuticle is constructed in the same plan in different parts of the body. In general it is made up of two layers, the primary and the secondary layer, which are distinctly recognized in the larva, but not so well in the pupa and adult. The staining reaction and resistance to KOH of these layers are different, as have been observed in other insects.¹⁾ The primary layer is deeply stained by haematoxylin, acid fuchsin and eosin, and is soluble in aqueous solution of KOH, while the secondary layer is easily stained by "Wasserblau," and is insoluble in KOH. From the resistance to KOH it can be admitted that chitin is a main constituent of the secondary layer, because of the existence of chitin in the cuticle of the silkworm, as already proved.²⁾

In the larval stage, a new cuticle begins to appear as a very thin membrane in the latter half of the course of moulting, which is composed of two layers. In spite of a thickness of about 0.01 mm. immediately after the fourth moulting, the cuticle grows rapidly for two or three days, and then very slowly for several days. A little before attaining the full-grown stage its growth becomes rapid again. In the full-grown stage it measures about 0.04 mm. in thickness (Fig. 2). In the course of its growth both the primary and secondary layers are found to thicken in similar manner, as may be seen from Fig. 1. In

---

1) Stegemann: Ist die Insektenkutikula wirklich einheitlich gebaut? Zool Jahrb. Abt. Anat. Ont., **50** (1929), 571-580.

2) Campbell: The detection and estimation of insect chitin; and the irrelation of "chitinization" to hardness and pigmentation of the *Periplaneta americana* L. Ann. Ent. Soc. Amer., **22** (1929), 401-426.

Fig. 1. Diagrammatic representations of larval cuticle in cross section of the thoracic part of the same group as in Fig. 2. $\times$ about 750. Blackened part represents the primary, and dotted part represents the secondary layer.

- a, b. 4th instar, near moulting. New cuticle (b) is formed under the old one (a).
- c. Exuvia of the 4th moulting.
- d. 3rd day of the 5th instar.
- e. Full-grown stage.

the early period of cuticle formation the cuticle of the pigmented part consists of two layers, the pigment and the secondary layer, but afterwards a normal primary layer appears between these two, to result in the formation of three layers, the pigment, the primary and the secondary layer. Therefore, the pigment production does not continue for a long while after the beginning of new cuticle formation, and soon follows the appearance of the same substance as the normal primary layer under the pigment layer. When attaining the prepupal stage, the worm body shortens to a certain extent by folding its skin. In sections the folded cuticle is found lined with an even "plasmatische Schicht." A pupal cuticle is not formed until a little while before pupation.

Immediately after pupation the cuticle is very thin, wholly devoid of pigment and consists mainly of the primary layer. However, the pigment makes its appearance during half a day, and the cuticle assumes an aspect similar to that of the larva. The arrangement of the layers is the same as in the larva in general, but it is often irregular, and pigmented parts are found scattered about in the

primary layer. The cuticle increases in thickness for three or four days, and then ceases to increase. Shortly afterwards the adult skin is formed under the pupal cuticle. It is found constructed in the same plan as the larval and the pupal skin, but it is more irregular, the cuticle chiefly consisting in some parts of the primary layer and in some others of the secondary layer.

In the exuviae of varying stages, the primary layer retains the condition almost similar to that before moulting, but the secondary layer is a great deal thinner (Fig. 1; a, c). The thinning occurs very near moulting, as has been reported by many authors.¹⁾

Increase of surface-area of body. To obtain some aspect of the volume augmentation of the cuticle in the larval stage, examination was made with the fifth instars from the newly moulted to the full-grown stage. After killing the larvae with boiling water, their cuticles were cut into several small pieces for applying to drawing papers. The cuticular parts were cut off and printed by blue print. After cutting off those printed parts, their weight was compared with that of the standard area. The results obtained are shown in Fig. 2. Multiplying the surface-area by corresponding thickness, the curve of volume augmentation was obtained (Fig. 2).

Increase of chitin (Fig. 3). The amount of chitin, which is a representative of the chemical constituents of the cuticle, was estimated by boiling the cuticle with 20% aqueous solution of KOH for 30 minutes. So far as experiments are concerned, chitin increases day after day in the fourth instar until just before the worms undergo moulting. In the fifth instar its increase continues as far as the worms

Fig. 2. Graph showing the growth of cuticle in the 5th instar, from newly moulted to full-grown stage.

1) Plotnikow: Über die Häutung und über einige Element der Haut bei den Insekten. Ztschr. wiss. Zool., 76 (1904), 333-366.

Fig. 3. Amount of chitin in worms from the 4th instar to adult.

feed, and ceases altogether, when they attain the prepupal stage. In the pupal stage chitin increases for the first three or four days, and then ceases to increase for several days, until it begins to increase again. This last increase is due to the formation of adult cuticle. In all cases, the exuviae contain very little chitin, only 10-20% of the cuticle before moulting. This fact coincides well with that the exuviae have a very thin secondary layer as already mentioned.

While in starvation, the increase of chitin also takes place, though very low at rate. For instance, the fifth instars, which contained on an average 0.0060 gr. of chitin on the second day, in spite of attaining 0.0137 gr. after feeding for two days, were found to contain only 0.0076 gr. after two days' starvation.

Conclusion. In the silkworm the cuticle augments always in volume, except in the prepupal stage, and chitin increases in like manner. The hypodermal cells produce chitin and other substances to form cuticle not only at the moulting period, but also continuously throughout the stages.