218. Pteridine Granules in Hypodermal Cells of the Silkworm Larva Causing Non-Transparency of Larval Skin^{*)}

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In the cytoplasm of larval skin of a normal silkworm strain, a large amount of pteridine granules (chromogranules) obstructing light penetration can be observed by optical microscope, and it is easily understood that their presence is the cause of non-transparent white larval skin. This observation was confirmed by electron microscopical studies of pteridine granules present in the larval skin cells or isolated from them. The present report deals with those observations and with the experimental results.

Materials and methods. Daizo, *w-c*, C-108, YD₄, and E-lemon were used as non-transparent and *w^{ol}* and *w^{ox}* as transparent strains.

Larvae with non-transparent skin were artificially made transparent by feeding them with mulberry leaves painted with 0.3% melamine solution.

Observations and experimental results. When a part of turbid cytoplasm of the larval skin was placed on a mesh covered with collodion and was electron-microscopically examined, a number of elliptical or spindle-shaped pteridine granules could be observed which were by electron-rays not completely penetrated.

As shown in Fig. 2 the pteridine granules in hypodermal cells of the normal strain are oval. The size of the granules is variable, but mostly it is $1 \times 1.5\mu$. In some pteridine granules the vesicle membranes can be clearly distinguished (Fig. 3).

In the cytoplasm of hypodermal cells no structures interrupting intense electron rays can be observed. Therefore, we may conclude that pteridine granules produced abundantly in the cytoplasm of larval skin cells of the normal strain are the bodies that make larval skin non-transparent.

Pteridine granules are abundantly formed immediately after each moulting stage as stated in our previous paper, namely, numerous small vesicles surrounded by ribosomal particles, 150–200Å in diameter, appear and the vesicles gradually increase in size. It seems that those structures are a kind of endoplasmic reticulum. The mature pteridine granules are formed by the growth and trans-

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formation of these precursors. During the growth of the granules, secretion products, pigment-polypeptides and uric acid-polypeptide complexes are secreted through the vesicular membrane into the vesicles and safely stored in them. Thus, in larval skin cells of the normal strain abundant pteridine granules are produced and packed compactly with various products.

The pteridine granules disintegrate after the loss of the contents of the granule vesicles in the following cases.

(1) A part of pteridine granules in larval skin cells disintegrate at each moulting stage. Namely, judging from our observation it seems that losing the normal function of vesicle membranes, the stored secretion products come out of the vesicles and then the

vesicle membranes disintegrate. Uric acid and pteridine compounds freed from the granules are probably discharged from the hypodermal cells into the body fluid together with decomposed polypeptides. According to Hatamura (1943) the amount of uric acid contained in the body fluid increased rapidly in the moulting stage, attaining the maximum value later. It is considered that this increase is due to the evacuation of granule contents.

Fig. 1. (a) Pteridine granules separated from fresh hypoderm of 50 larvae at 5th day of 5th instar (before the maturation stage) (b) those separated from larvae at a later maturation stage.

integration of pteridine granules in hypodermal cells begins during the maturation stage. At first the contents of the granule vesicles are rapidly lost and the vesicle membranes remain. Often a mottled pattern, probably formed by folds in the vesicle membranes can be observed (Figs. 4, 5). Disintegration and disappearance of those vesicular membranes occurs from later maturation stage to prepupal stage.

Since the density of pteridine granules is remarkably reduced

(2) At the end of the completed larval stage the larval skin becomes semi-transparent. This period will be tentatively given the technical term "maturation stage". The maturation stage of Daizo or *w-c* strain ranges from 5 to 10 hours at 25°-27°C. Dis-

by the loss of the vesicle contents, those pteridine granules can hardly be isolated by the usual method described in the previous paper (Tsujita and Sakurai 1966). Namely, a large amount of pteridine granules can be gathered from fresh hypodermis of 50 larvae at 5th day of 5th instar (before the maturation stage) as shown in Fig. 1a, but only a small amount of granules can be separated at later maturation stage (Fig. 1b) and granules can be hardly separated at its end owing to the loss of vesicle contents. It seems that pteridine compounds as well as uric acid freed from granules together with decomposed polypeptides are discharged from the hypodermal cells into the body fluid. This consideration may be supported by Hatamura's finding (1943) that uric acid contained in the body fluid attained the largest amount at the maturation stage.

(3) The fact that disintegration of pteridine granules can be artificially induced had been reported (Tsujita and Sakurai 1963). Disintegration and regeneration processes of the granules were electron microscopically observed.

When mulberry leaves painted with 0.3% melamine solution were continuously fed to normal or lemon larvae (yellow larvae) from the beginning of their instar, disintegration of pteridine granules occurred after 2-3 days after the treatment; the contents of granule vesicles were lost through vesicle membranes (Fig. 6 a, b, c) which disintegrated and disappeared.

By disintegration of all pteridine granules, the pteridine compounds and uric acid are lost from larval skin cells, and all larvae treated with melamine become highly transparent. Artificially produced transparent hypodermis changed into normal opaque, when the larvae were fed for about two days with fresh mulberry leaves without melamine. In this recovering process, first a number of minute particles appeared in the cytoplasm and gradually increased in size. Fig. 7 shows these small particles, almost uniform in size, during the recovery process from the melamine treatment. Although the skin of larvae in this stage is semi-transparent, the larvae show white, non-transparent skin, when those minute particles grow to usual size.

When mulberry leaves painted with 0.3% melamine solution were continuously fed to larvae homozygous for *w-3*, *w-b*, or *ol* whose hypodermis is weakly or moderately transparent, disintegration of pteridine granules occurred one day after the treatment, although it takes more than 2 days for the melamine treatment of normal larvae as stated before. It seems that this reduction of the period which is necessary for disintegration of the granules is due to

Figs. 2-7.

defective granules of the mutant larvae.

Discussion. Larvae of the normal silkworm are opaque owing to the presence of a large amount of pteridine granules containing uric acid and pteridine compounds in the hypodermal cells. Jucci (1932) and Shimizu (1943) had clarified the relation between non-transparency of the larval skin and the content of uric acid in its cells. According to them, normal silkworm larvae have non-transparent skin because a large amount of crystal-like clusters of uric acid are contained in their skin cells, while the amount of these bodies is reduced in the hypodermis of mutant larvae with transparent skin. Later, Aruga (1950) reported that uric acid is found in the spherical granules of larval skin. Hama and Fukuda (1964) reported that in the silkworm (wild and lemon) the pteridine granules are of oval but rather oblong shape ($\text{ca } 0.5 \times 0.25 \mu$).

We could not find any crystal-like clusters of uric acid in the cytoplasm of fresh larval skin cells. Besides, according to our studies, uric acid as well as pteridine compounds are contained in the same granules which were designated by us as "pteridine granules". Therefore, it seems that uric acid clusters and the granules studied by several authors mentioned above are probably identical with our pteridine granules.

It is assumed that the rapid loss of contents of pteridine granules during the maturation stage is probably due to the loss of normal function of the granule membrane. Although the mechanism of disintegration of the granules which is artificially induced by melamine treatment has not been clarified, it is supposed that the granule membrane is broken by the treatment.

As stated before each pteridine granule consists of a vesicle membrane and secretion products.

According to the general belief, it seems that the hypodermal cells of the silkworm larvae have the ability to absorb uric acid

Figs. 2-7. Pteridine granules in larval skin cells. $\times 20,000$

Fig. 2. Pteridine granules in hypodermal cells of a *w-c* larva; they are elliptical and of almost uniform size.

Fig. 3. Pteridine granules in hypodermal cells of a YD_4 larva; granule vesicle membranes can be discriminated.

Fig. 4. Pteridine granules in hypodermal cells of a YD_4 larva at maturation stage. The contents of granule vesicles are lost and a mottled pattern can be observed on the vesicle membranes.

Fig. 5. Pteridine granules in hypodermal cells of a YD_4 larva at maturation stage. The contents of granule vesicles are lost and a mottled pattern can be seen on the vesicle membranes. A part of granule vesicles is disintegrated.

Fig. 6 a, b, c, show disintegrating granules after melamine treatment.

Fig. 7. Several minute granules appearing in the recovery process from melamine treatment.

from the body fluid and to store it within the cells.

According to T. Hama and S. Fukuda who tried reciprocal skin transplantation between lemon and wild strains, the pteridine pattern in hypodermal cells of lemon and wild strains is perfectly host-dependent, that is lemon skin becomes white and wild skin becomes yellow. On the other hand, in one of our experiments the activity of enzymes concerned with pteridine metabolism such as pterine dehydrogenase or pterine reductase in larval adipose and hypodermal tissues was examined (Tsujiita and Kojima, unpublished). The results indicate that the activity of those enzymes is very strong in adipose tissue, but weak or deficient in hypodermal tissue.

Nevertheless, products of pteridine metabolism, such as isoxanthopterin and sepiapterin are abundantly accumulated in the hypodermis, but they can hardly be detected in the adipose tissue of larvae. In addition, experimental results concerning the incorporation of labeled amino acid into pteridine granules suggest that polypeptides are synthesized on the vesicle membranes surrounded by ribosomes (Sakurai and Tsujita 1966).

On the basis of those experimental results the following hypothesis is presented for the formation of pteridine granules. The hypodermis has the ability to absorb uric acid as well as pteridine compounds through the body fluid from other organs in which those compounds are produced; those compounds combining with polypeptides produced around the granules are secreted into granule vesicles and stored in them. In the hypodermal cells of normal larvae the granule vesicles have complete or sufficient ability to secrete and store those products and so abundant pteridine granules are produced. However, in the hypodermal cells of mutant larvae homozygous for w^{ox} , or w^{ol} , this ability is defective and no mature pteridine granules or a small amount of minute granules is produced, as described in another paper.

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