

## Embryonic Studies on the Fluorescent Compounds in Silkworms

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The fluorescent compounds exist widely in various organisms, and some of them play an important role physiologically. In silkworms there exist flavin, pterin and many other fluorescent compounds. It is thought partly due to the mulberry which they feed on but mostly due to the substances which are compounded or resolved in their bodies. As for the fluorescent compounds of insect eggs, there are the study on flavin and pterin by Bodine (1947, 1948), and the report on the process of tryptophan system substance in silkworm eggs by Yoshikawa. Ariga also observed that there exist riboflavin, 3-hydroxykinurenine, kinurenine and pterin sort as the fluorescent compounds in silkworm eggs, the skin of larvae, blood, chrysalis and ants. But these studies are proceeded by means of the chemical extraction.

We investigated the fluorescent compounds in silkworms, especially that in malpighian tube embryologically through fluorescence microscope.

### MATERIAL AND METHOD

The silkworms we afforded to the test are Hi No. 123 × SHI No. 123. We extracted the materials at every stage between the 1st and the 40th day after incubation, and at the same time we fixed them in 10 percent formalin solution. After this, we made paraffin sections and stained with acridin yellow and acridin orange, and examined under the fluorescence microscope. For all materials, we made the specimen, stained with haematoxylin-eosin, and non stained just for the purpose to compare each other.

### EXPERIMENTAL RESULTS AND CONSIDERATION

On the 1st day (30 mm in length) the malpighian tubes are seen in a row around the gut. The cavity of the gut is round and no secretion is seen in the cavity. On the 3rd day (4.0 mm in length) the malpighian tubes grow larger and a little secretion of light purple is recognized in the cavity (Fig. 2). Next on the 8th day (8.2 mm in length) the cavity is perfectly filled with the secretion of light purple. On the 9th (8.5 mm in length) the secretion partly looks granule (Fig. 3). On the 14th day (1.6 cm in length) the cavity is perfectly filled with the granular substance (Fig. 4). On the 17th day (1.9 cm in length) in the cavity, needle-like crystal of light

purple is recognized. On the 21st day (2.6 cm in length) cavity is perfectly filled with the needle-like crystal (Fig. 5 & 6). After this, this condition is continued till they get into the former stage of changing pupal, meanwhile any remarkable change is not seen in crystal form and color etc. With 1% toluidin-blue, the malpighian tubes are stained red-blue deeply.

The non-stained specimen under the fluorescence microscope shows that granules appear in the secretion of the malpighian tubes on the 9th day after incubation. This granule issues a peculiar fluorescence of very strong blue. The epidermis is light yellow, and the dermic layer has rather light yellow color.

We treat the malpighian tubes for a minute A.G. 1/2000 solution, and study it under the fluorescence microscope. The wall of malpighian tube assumes a yellow color. The epidermis assumes a light yellow green color, dermic layer has a tone of yellow color. On investigation of the gut, the chorion has a yellow in color, a mucous membrane, a green yellow in color, and muscular layer and adipose tissue, a light yellow color.

We treat the malpighian tubes for ten minutes with A.O. 1/2000 solution, and examine it. The epidermis assumes a light green yellow color, dermic layer has a tone of dark grey. On investigation of the gut, the chorion is dark grey, the mucous membrane yellow green, and the muscular layer and adipose tissue dark yellow. According to the organization chemical detection method, we treat it with 1% hydrochloric liquor that is added with a little zincic powder and peroxide of hydrogen. But in this case riboflavin is not noticed as a blue granule as it should be.

It is already known that riboflavin exists in the eggs of silkworms, and in the malpighian tubes of larva as needle-like crystal. And riboflavin is smoothly made by the secretion of malpighian tubes and ecdysis gland in case of ecdysis. About 3 days after incubation, it starts the function of a gland gradually, and is thought to put forth secretion into the cavity.

## CONCLUSION

On the specimen, in each stage of metamorphosis from incubation hence to the former stage of changing pupal, we examined the fluorescent compounds and especially the fluorescent compounds of malpighian tubes embryologically under the fluorescence microscope.

In the malpighian tubes of silkworms, the granule, on which a blue inherent fluorescence was observed, is put forth in the secreted substances on the 9th day after incubation.

The treatment of this granule with fluorescent pigment (acridin-yellow or acridin-orange) causes a light blue fluorescence. This granule grew and crystallized in needle-like plate on the 21st day after incubation.

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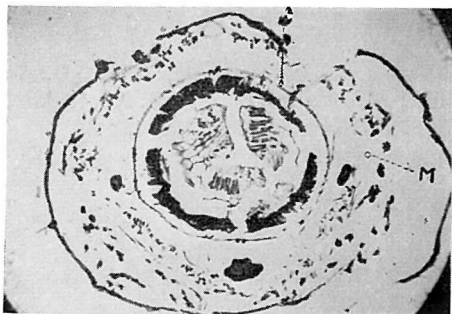


Fig. 1



Fig. 4

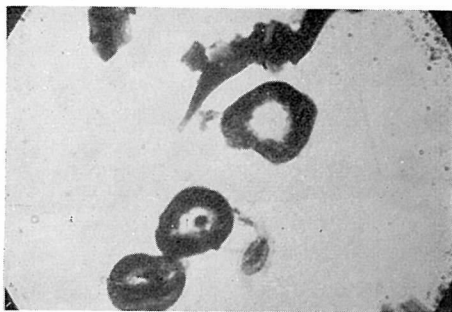


Fig. 2

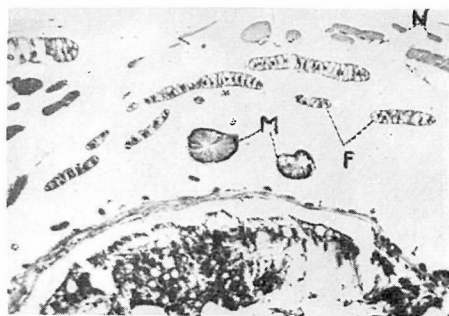


Fig. 5

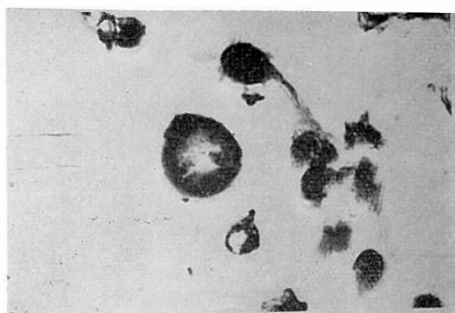


Fig. 3

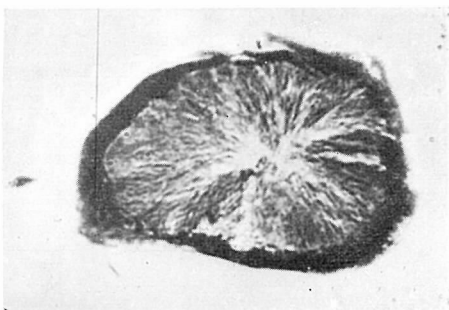


Fig. 6

- Fig. 1. Incubation hence the 1st day (3.0 mm in length)  
Cross section of a silkworm. M.....malpighian tube.
- Fig. 2. The 3rd day (4.0 mm in length).  
Secretion is noticed in the malpighian tubes.
- Fig. 3. The 9th day (8.5 mm in length).  
Granules are noticed in the malpighian tubes secretion.
- Fig. 4. The 24th day (1.6 cm in length).  
The malpighian tubes are quite filled up with granules.
- Fig. 5. The 21st day (2.6 cm in length).  
Needle-like crystal is noticed in the malpighian tubes.  
F.....Adipose tissue M.....Malpighian tubes N.....Muscular layer
- Fig. 6. The 21st day. Enlargement of the same above.