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# Fine Structure of Median Neurosecretory Cell in Diapause and Non-Diapause Brains in the Silkworm, *Bombyx mori*

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**Summary**

The electron micrographs of the larval brain of the silkworm, *Bombyx mori.* show that median neurosecretory cell of diapause-egg producer may participate in the production of lipo-granules and that of non-diapause egg producer may do in the production of electron-translucent vesicles.

It was found that ribosome-like particles of diapause-egg producer gradually developed into highly dense particles and came into line along the smooth-surfaced endoplasmic reticulum. They finally became lipo-granules.

## INTRODUCTION

During the course of an experiment on diapause of the silkworm, *Bombyx mori.* PARK and YOSHITAKE (1971) found that in the diapause-factor cells located in the suboesophageal ganglion, there was a difference in the rough-surfaced endoplasmic reticulum, in the process of cytoplasmic granule formation, and in the amount of cytoplasmic granules between producers of diapause and non-diapause eggs. PARK(1973) proposed the diapause-regulator producing cells may give some information to the diapause factor cells in the suboesophageal ganglion.

FUKUDA(1951, 1952) assumed that the voltinism may be regulated by the pupal brain, which controls the secretion of the suboesophageal ganglion. When the brain completely suppresses the secretion of the suboesophageal ganglion, moths lay non-diapause eggs and when the brain promotes it, they lay diapause eggs.

NISHIITSUTSUJI-UWO(1961) reported that in *Bombyx mori*, the perikaryon of the neurosecretory cell in the pars inter-cerebralis contains characteristic granules in addition to the common features of the nerve cells, and the mitochondria in the Golgi zone.

BASSURMANOVA and PANOV(1967) reported that in the *Bombyx mori*, the large cells identified with median neurosecretory cells contain largely electron-lucent vesicles and scanty electron-dense granules, and there is no difinite evidence regarding the origin of the electron-lucent vesicles. To the best knowledge of the authors, no one reported the reaction of median neurosecretory cells to high and low temperature experienced by the eggs during incubation.

Accordingly, the present paper refers to the fine structures of median neurosecretory cells of brains of diapause and non-diapause silkworm, *Bombyx mori.*

## MATERIALS AND METHODS

The female *Bombyx mori* of the bivoltine Daizo strain destined to lay diapause and non-diapause eggs was used. The brain-suboesophageal ganglion complex at the 5th instar of larvae was taken out under a dissecting microscope.

The brain-suboesophageal ganglion complex was

fixed for 2 hr in 5% glutaraldehyde in 0.2M cacodylate buffer, washed with four changes of cacodylate buffer (0.05M) plus sucrose(0.34M) for about 15 hr, post-fixed for 2 hr in 1% osmium tetroxide in 0.2M cacodylate buffer, dehydrated in an ethanol series, and embedded in Epon 812. Sections were double-stained in uranyl acetate and lead acetate(SATO's (1968) modified lead staining method).

## RESULTS

The largest median neurosecretory cells in the larval brain of *Bombyx mori* could be easily identified with their size and conspicuous feature of cytoplasm on the basis of the identification of BASSURMANOVA and PANOV(1967).

Median neurosecretory cells both in diapause and non-diapause brains were with nuclei of irregular shape and prominent nuclear process in Fig. 6. The appearance of the diapause median neurosecretory cells was different from that of non-diapause ones as shown in Fig. 1 and 5.

The Fig.1 and 2 show numerous well differentiated Golgi apparatuses which are not so much related to the production of secretory granules in diapause median neurosecretory cell, but the Fig.5 shows a few Golgi apparatuses which have close relation to the formation of secretory granules in non-diapause one (See Fig. 7).

In the diapause larva, it is possible to distinguish at least two types of neurosecretory granules, one is electron-dense neurosecretory granules and the other one is electron-translucent neurosecretory granules ranging from 100 mμ to 200 mμ in diameter. Almost all of granules of diapause median neurosecretory cell are covered with slightly swelled rough-surfaced endoplasmic reticulum. They are free in the lumen of rather extended tubular and well developed cisternal profiles of the granular reticulum. Slightly swelled endoplasmic reticula are filled throughout cytoplasm of median neurosecretory cells. Cisternae of endoplasmic reticula, especially along the cell periphery, are very swollen(See Fig. 2), with the electron-dense and electron-lucent neurosecretory granules.

In the Fig. 3 and 4, it is clear that in median neurosecretory cell of diapause brain, one more type of specific granules are being produced besides two types of neurosecretory granules as mentioned above. They are larger than ribosomes. They gradually developed into highly dense particles, which came into line along the smooth-surfaced endoplasmic reticulum. They are called ribosome-like particles. These conjugated into a small group which was covered with smooth-surfaced endoplamic reticula.

Furthermore a larger group consisting of those small groups was also covered with the smooth-surfaced endoplasmic reticulum in limited area of cytoplasm of neurosecretory cell. Authors can suggest that the largest median neurosecretory cells of diapause-egg producer participate in the formation of lipo-granules by smooth-surfaced endoplasmic reticulum.

In the Fig. 5, the median neurosecretory cells of the brain of non-diapause egg producer are entirely filled with numerous well differentiated rough-surfaced endoplasmic reticula. There are two kinds of round shape granules, such as the electron-dense granules and some round cisternae of the granular endoplasmic reticula. These round cisternae are considered to be electron-translucent neurosecretory vesicles with a diameter of 150–400 mμ as secretory formations specific for median neurosecretory cells of non-diapause egg producer. Authors did not observe the secretion of electron-translucent vesicles, but BASSURMANOVA and PANOV (1967) reported that electron-lucent vesicles, like electron dense granules, go along the axon from the cell body into axon terminals situated in the corpus allatum. Electron-translucent vesicles were largely centered near area around the nucleus, especially along the nuclear process. The nucleolus seemed to be very active (See Fig. 5) and nuclear process was very prominent (See Fig. 6).

It was clear that the rough-surfaced endoplasmic reticulum of diapause median neurosecretory cell was different from that of non-diapause one. In the former case it showed to produce very swollen cisternae of rough-surfaced endoplasmic reticula with free electron-translucent and electron-dense granules, but in the latter case it participated in the production of electron-translucent vesicles.

## DISCUSSION

Agranular reticula are described in the gunea pig;

adrenal cortex cells which participate in the synthesis of steroid hormones (SHERIDAN and BELT, 1964). The authors' findings that diapause median neurosecretory cell formed very swollen cisternae of rough-surfaced endoplasmic reticula which contained free electron-dense or electron-translucent granules and both, and non-diapause median neurosecretory cell produced electron-lucent vesicles rest on the reaction of median neurosecretory cell to light and temperature during incubation at egg stage. PARK and YOSHITAKE(1969) reported that much of the labelled glycine and uridine was incorporated into the brain and the suboesophageal ganglion on the third day after blastokinesis in the embryos incubated at 25°C, and at 17°C the radio-autographic grains were distributed uniformly, with no localization in the brain and the suboesophageal ganglion. Authors are inclined to consider the electron-lucent vesicles as secretory formations specific for non-diapause median neurosecretory cell which may participate in the synthesis of steroid hormones as proposed by SHERIDAN and BELT(1964). The release of these vesicles was not observed in non-diapause specimens but discharging all the granules was noted in diapause ones.

As mentioned above, median neurosecretory cell was rich in electron-lucent vesicles when the silkworm eggs were incubated at a low temperature and under dark condisions. In this connexion, ÖZTAN(1966) also suggested that electron-lucent vesicles in *Zoarces viviparus* are particular neurosceretory products, and different from electron-dense granules. When the silkworm eggs were incubated at a high temperature and under illumination, slightly swelled endoplasmic reticulum with some of granules could be seen and sometimes there occurred very swollen cisternae of endoplasmic reticula along the cell membrane. It is difficult to see how this difference of diapause and non-dipause egg producers can be related to function of diapause-factor cell (PARK and YOTHITAKE, 1971) and diapause-regulator cell (PARK, 1973) of suboesophageal ganglion of the silkworm necessary for the determination of voltinism.

In this experiment, the ribosome-like particles wrapped up with smooth-surfaced endoplasmic reticulum seem to be a ground substance for the production of lipo-granules of median neurosecretory cells of diapause-egg producer. These ribosome-like particles are present only in median neurosecretory cells of the diapause-egg producer. The question of the origin of ribosome-like particles has not been solved yet. One of the possible ways is formations by the function of smooth-surfaced endoplasmic reticulum.

Electron-dense granules in both diapause and non-diapsuse median neurosecretory cells are very scantier than those in other kinds of neurosecretory cells of the brain in the silkworms. A more detailed account of these related experiments will be reported in a subsequent paper.

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## 摘 要

## 家蠶의 休眠性 및 非休眠性 腦의 腦間部 神經分泌細胞의 微細構造에 관한 硏究

家蠶 幼虫의 腦에 대한 電子顯微鏡的 觀察에 의하면 腦間部의 最大神經分泌細胞는 休眠卵性에서 脂肪性 顆粒을 生成하고 非休眠卵性에서는 半透明體의 電子小胞를 生成하고 있었다.

休眠卵性에서 처음 리보좀으로 보이는 粒子들은 서서히 電子密度가 높은 粒子들로 發展하면서 滑面 小胞體에 連하여 排列되고 마침내 이 粒子들은 脂肪性顆粒으로 된다.

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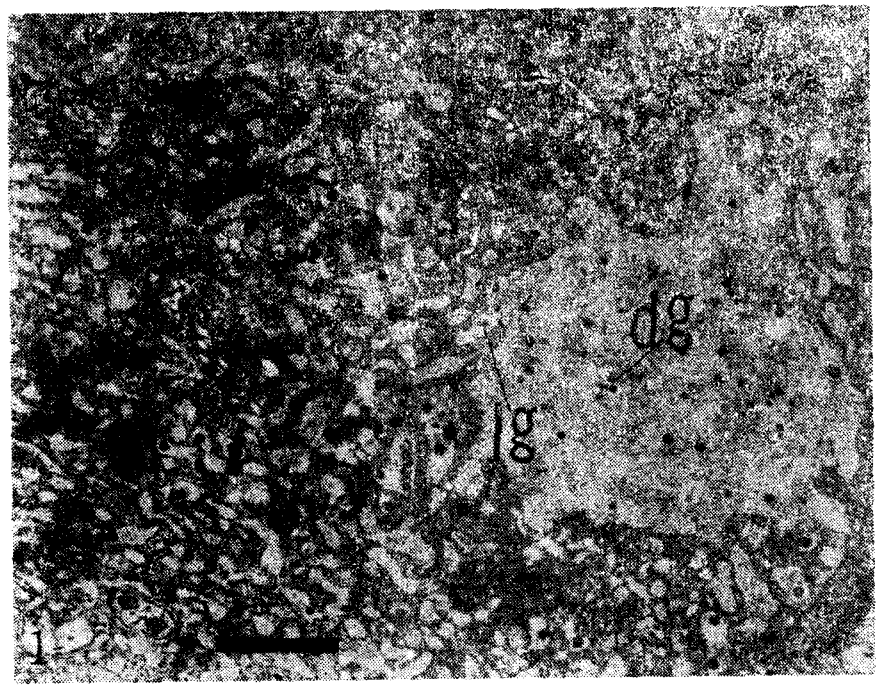


**Fig. 1.** Electron-micrograph showing numerous Golgi apparatuses(g), electron-dense(dg), electron-lucent granules(lg), and swollen endoplasmic reticula in diapause median neurosecretory cell. scale=2$\mu$


**Fig. 2.** Electron-micrograph showing very much swollen endoplasmic reticula along the cell membrane with free electron-dense(dg) and electron-lucent granules(lg). scal=0.5$\mu$

**Fig. 3.** Electron-micrograph showing ribosome-like granules(r) largely centered in cytoplasm of diapause median neurosecretory cell, scale=0.5$\mu$

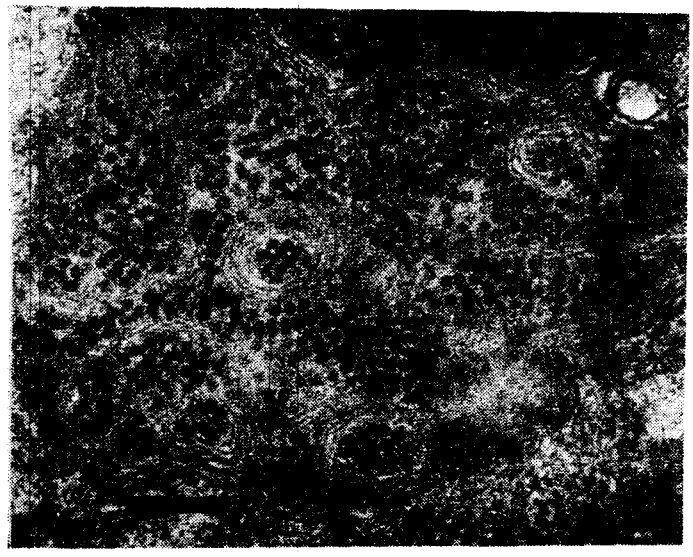

**Fig. 4.** Electron-micrograph of ribosome-like granules (r) wrapped up with by smooth-surfaced endoplasmic reticula. scale=0.25$\mu$

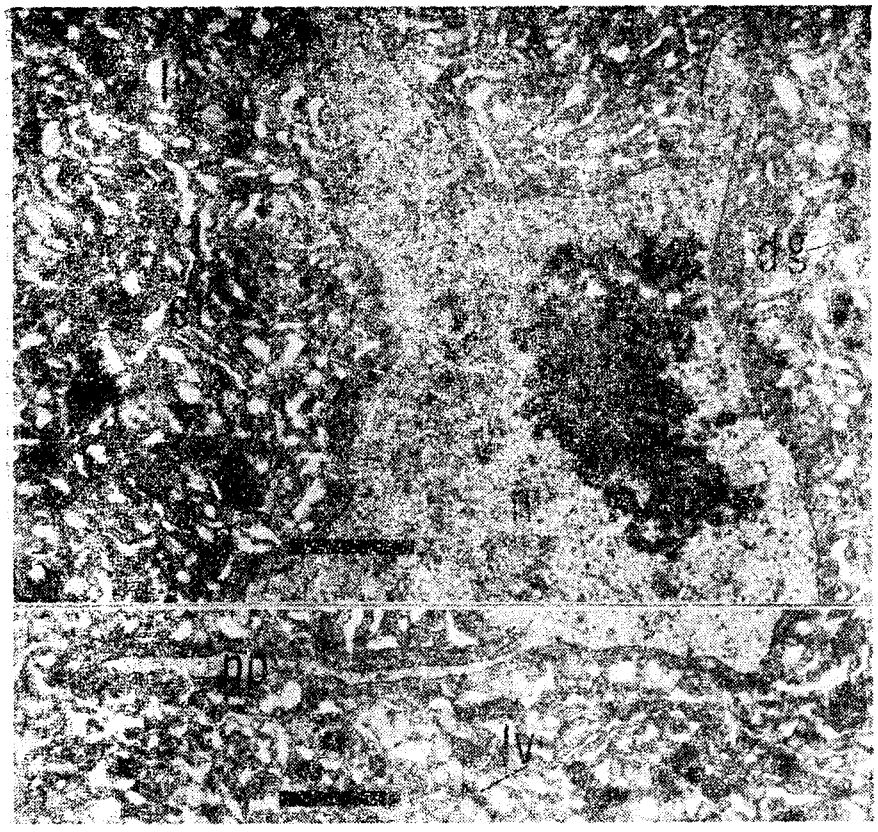

Fig. 5. Electron-micrograph of non-diapause median neurosecretory cell. lv, electronl-ucent vesicle; dg, electron-dense granule; er, endoplasmic reticulum. scale=2$\mu$

Fig. 6. Electron-micrograph showing electron-lucent vesicles(lv) near the nuclear process. scale=2$\mu$

Fig, 7. Electron-micrograph showing electron-dense granule formation in Golgi apparatus(g). scale=0.5$\mu$