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On the Effects of Juvenile Hormone and Ecdysterone for the Parthenogenetic Occurrence in the Silkworm, Bombyx mori L. (Lepi-d optera :Bombycidae)

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The morphological change of an egg cell and the manifestation state of a parthenogenetic egg were investigated by administering Juvenile hormone and ecdysterone being endocrine hormone at a pupal stage. The results obtained are as follows.

1) The manifestation rate of a parthenogenetic eggs was highest in an ecdysterone administration group and lowered in a control group and became lowest in a juvenile hormone administration group.

2) In view of the morphological change of an egg cell, the microvilli formed between an oocyte and follicle cell was most markedly developd in the ecdysterone administration group but the formation of microvilli was contrarily suppressed in the juvenile hormone administration group.

3) The amount of female specific protein stored in an eggs was also much in the ecdysterone group but contrarily had a tendency to decrease in the juvenile hormone group. From the foregoing, It was considered that the degree of a parthenogenetic rate was determined on the basis of the amount of intraovaular protein regulated by endocrine hormone.

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INTRODUCTION

Insect eggs are matured by such a mecanism that vitellogenin synthesized in a fatbody is taken in eggs through the folicle cell by pinocytosis phenomenon (Roth and Porter, 1964. Elfer, 1965. Patchin and Devey, 1968). In general, the fertility of eggs is closely connected with the maturity thereof and obtained at the metaphase of the first meiotic division (Barros and Munoz, 1973). In panstrongylus megistus, it was already reported that prothoracic gland hormone was required in this meiotic division (Furtado, 1979). As mentioned above, the fertility of eggs is obtained as the result of the connection with various factors. The parthenogenesis of the eggs of domesticated silkworm is brought about by polar body fetilization (Sato, 1934) and different in its mecanism from general fertilization. Therefore, even in the case of the parthenogenesis of the eggs of domesticated silkworm, it is questionable whether the action of endocrine hormone is same as the case of general fertilization. Hereupon, for the purpose of elucidating these problems, this experiment was conducted.

MATERIALS AND METHODS

Materials Tested and Hormone Treatment: As race tested, Ohl6, Kojiki, Nichi 1, Nichi 124, Ohkusa, shungetsu, Shi 108, Shi 124 and Hoshow were employed. Manta (Ohtsuka Seiyaku K. K.) was used as juvenile hormone and ecdysterone (Roto Seiyaku K. K.) was used as molting hormone and both of them were respectively injected in an amount of 5 μg/pupa 2days and 4days after pupation. The number of the ovarian eggs used in this treatment were respectively 20 moth.

Induction Treatment of parthenogenesis: Experiments were performed according to the method of Astaurov (1967) as follows: As soon as after moth emergence, ovarian eggs were obtein by dissecting female moth and heat- treated at 46℃ for 18 min in a water bath. After the treatment, they were incubated at 18℃ for 3 days and then preserved at 25℃. The number of eggs which showed apparent dark pigmentation, a sign of embryonal development, was counted and the ratio of dark pigmentary eggs to total eggs was expressed as the percentage of parthenogenetic eggs.

Preparation of Electron-Microscopic Specimen Eggs: Eggs being ovarian ones 6 days after pupation but almost degenerated in nurse cells were used and immobilized according to usual method. That is, said eggs were subjected to preimmobilization using 5% glutaraldehyde (buffered with 0.1M sodium cacodylate) at 5℃ for 48 hr in a refrigerator and subsequently

washed four times with a 0.05M sodium cacodylate buffer solution. Postimmobilization was performed at room temperature for 3 hr using 1% osmium tetraoxide (buffered with 0.1M sodium cacodylate). The immobillized eggs were dehydrated by ethanol/acetone series and subsequently embedded in an epoxy resin. An ultra-thin section for an electron microscope were prepared by Hitachi MUM type 2 Ultratome. The ultra-thin section was stained doubly with an uranyl acetate/lead staining bath (Sato, 1968). The used electron microscope is HU-12Type 12A (Hitachi Seisakusho).

Polyacrylamide Gel Electrophoresis: Electrophoresis using a 7.5% polyacrylamide gel as a support was performed according to a Davis' method (1964). 0.05g of eggs were ground in a physiological saline solution to be centrifuged at 2000 rpm for 20 min while 10 μl of the obtained supernatant liquid was superposed on a specimen layar to be subjected to electrophoresis for 60 min under a constant current of 2 mA/a column. After the obtained specimen was immobilized by 10%TCA, said specimen was stained using amido black 10B and decolored with acetic acid to be measured by a densitometer (Fuji Riken Type AD-IV).

RESULTS AND DISCUSSION

I. Inter-strain Difference in the Percentage of Parthenogenetic Eggs: Examination of the inter-strain difference was performed by treating 8 original races, 7 hybrids, and reciprocal cross hybrids for their parthenogenesis induction. As shown Fig. 1, the percentage of parthenogenesis among original races varied greatly, ranging from 25.3% in shi 108 to 91.5% in Ohkusa. This showed that the occurrence of parthenogenesis is controlled genetically. When the percentage of parthenogenesis in the same race was correlated to silkworm rearing seasons, it was lower in every race reared in late autumn than in spring, with a positive difference at the 1% significance level.

The cause of decrease in the percentage of parthenogenesis in late autumn rearing may be attributed to seasonal differences in the rearing temperature and quality of mulberry leaves with both variables inducing parthenogenesis earlier in the season.

Fig. 2 shows the results of examination on the percentage of parthenogenesis in the original races and hybrid strain. In the F_1 hybrids (except for shi 108 X kojiki) the percentage was high (76-95%), and heterrosis was observed. When the results were correlated to crossing and reciprocal crossing, an increasing tendency in percentage was observed in hybrids obtained from a mother moth having a high percentage of parthenogenesis. This also shows that maternal factors influenced the occurrence of parthenogenesis. Similar results were also observed by sugai et al.(1983). and Takei et. al.(1990).

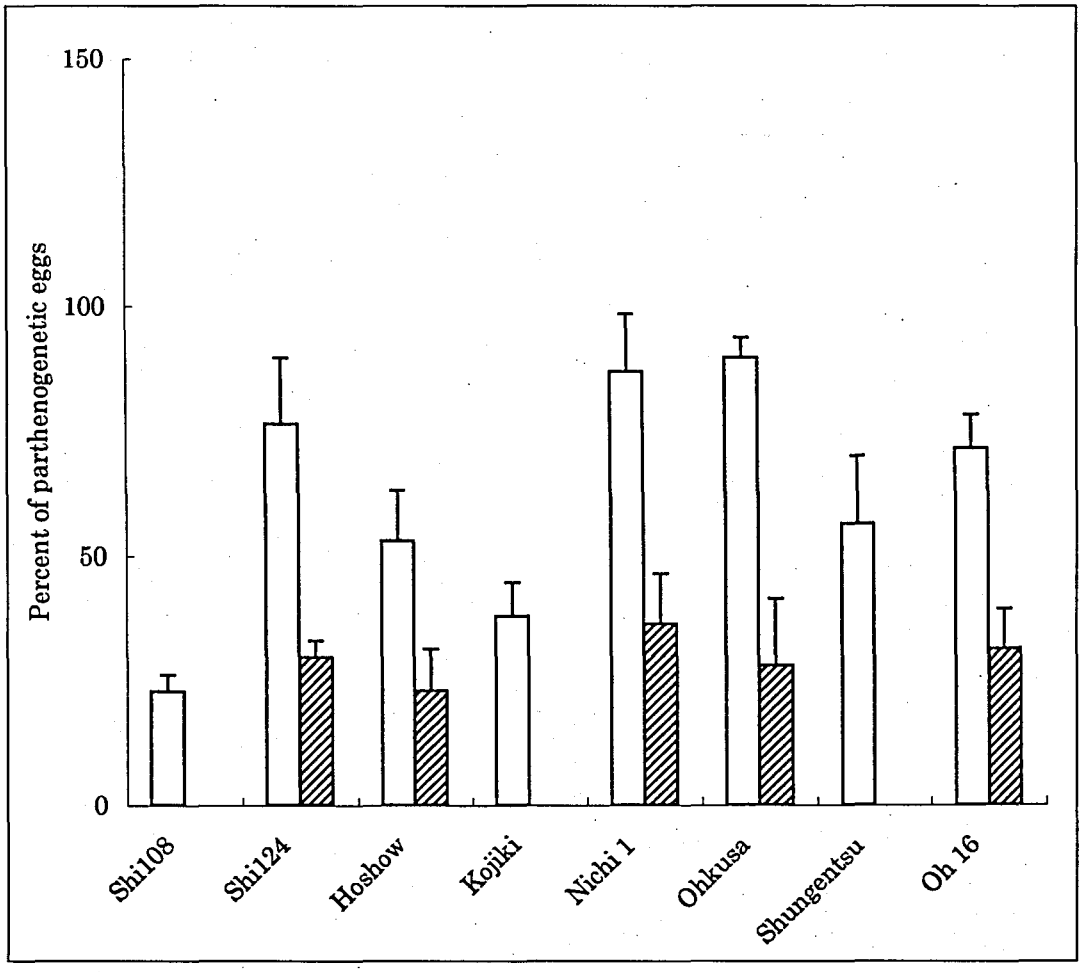

Fig.1. Racial difference in percent of parthenogenetic eggs. Open and hatched bars represent spring and autumn rearing seasons. T-marks represent standard deviation.

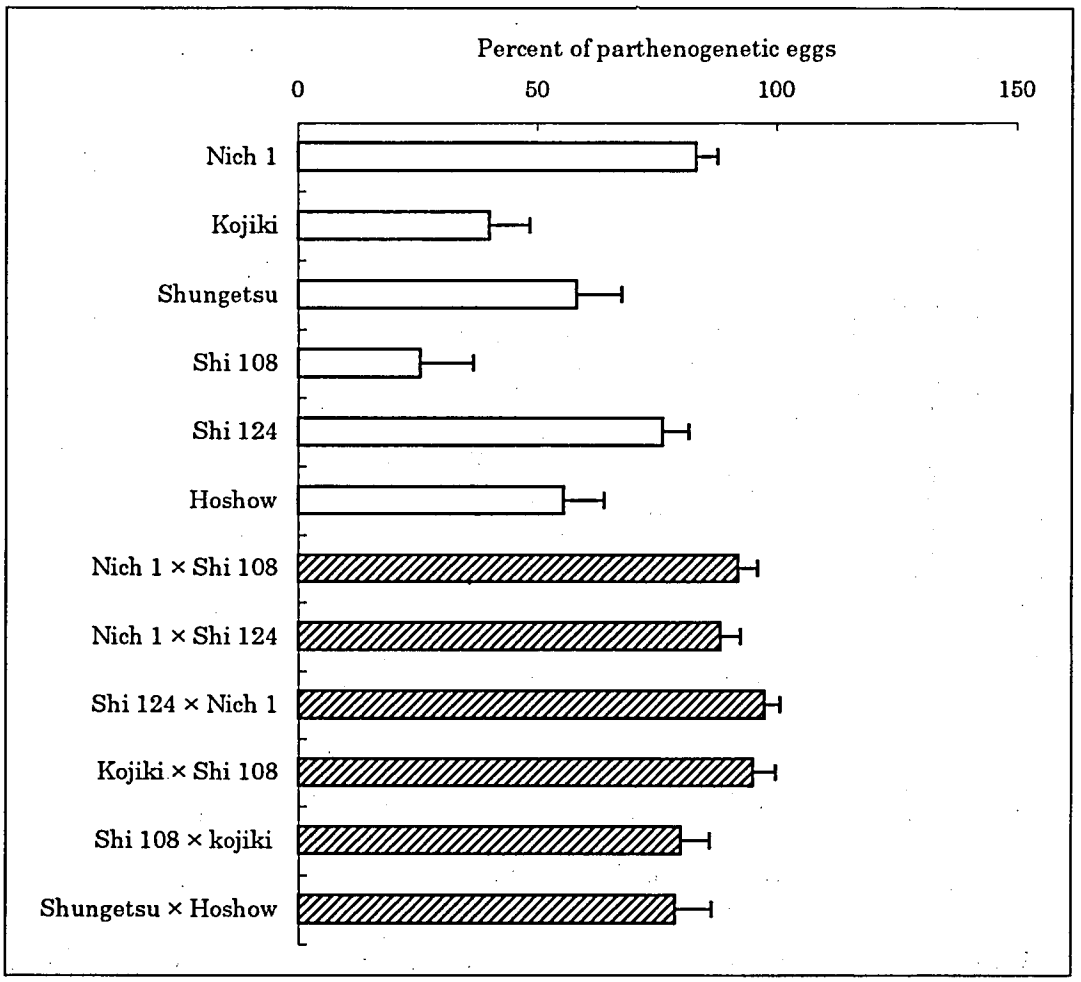

Fig.2. Racial difference in percent of parthenogenetic eggs. Open and hated bars express original races and hybrids.

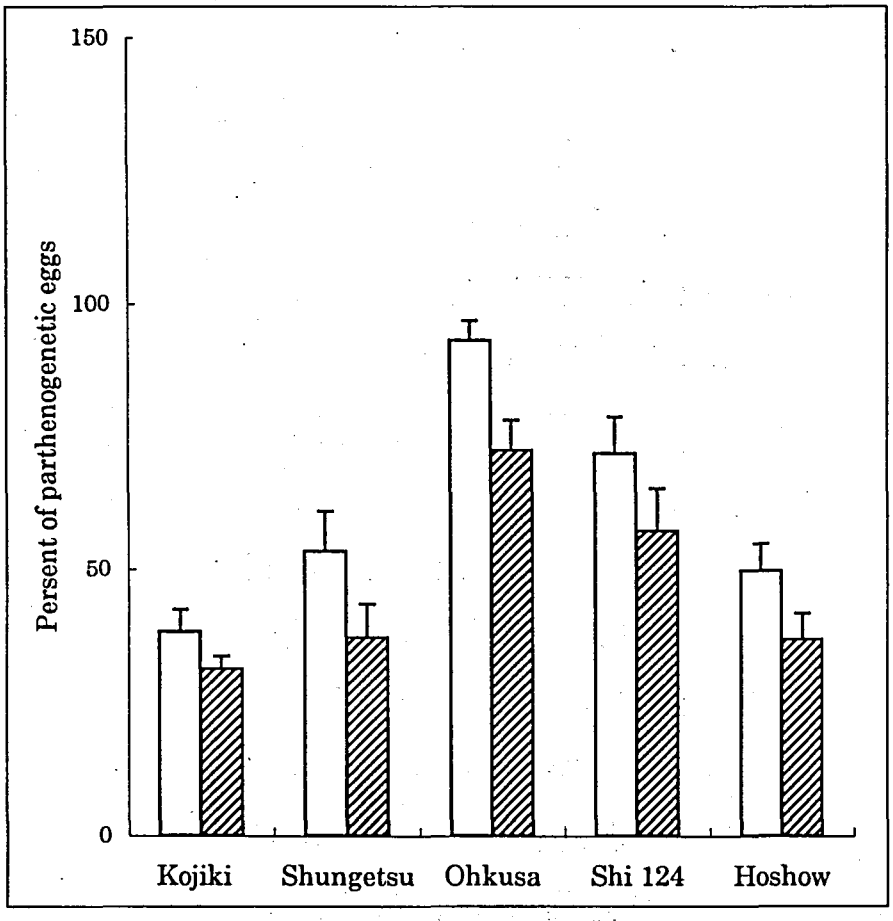

Fig.3. Racial difference in percent of parthenogenetic eggs treated with juvenile hormone in a pupal stage. Open and hatched bars represent a non-injected(control)and juvenile hormone injected eggs. T-mrarks represent standard deviation.

II. Effect of Juvenile Hormone on Manifestation of parthenogenetic Eggs: The comparative investigation was conducted between the parthenogenetic rate of the hormone administration group and that of the control group in each races. The difference is confirmed between both groups in all of the races, when juvenile hormone was administered, a parthenogenetic rate is clearly lowered as compared with the control group and the manifestation of parthnogenesis is suppressed (Fig. 3).

III. Effeet of Ecdysterone on Manifestation of parthenogenetic Eggs: ecdysterone was injected in an amount of 5μg/pupa 2 days and 4 days after pupation. A parthenogenetic egg rate was calculated about a month after parthenogenetic treatment to obtain the results shown by

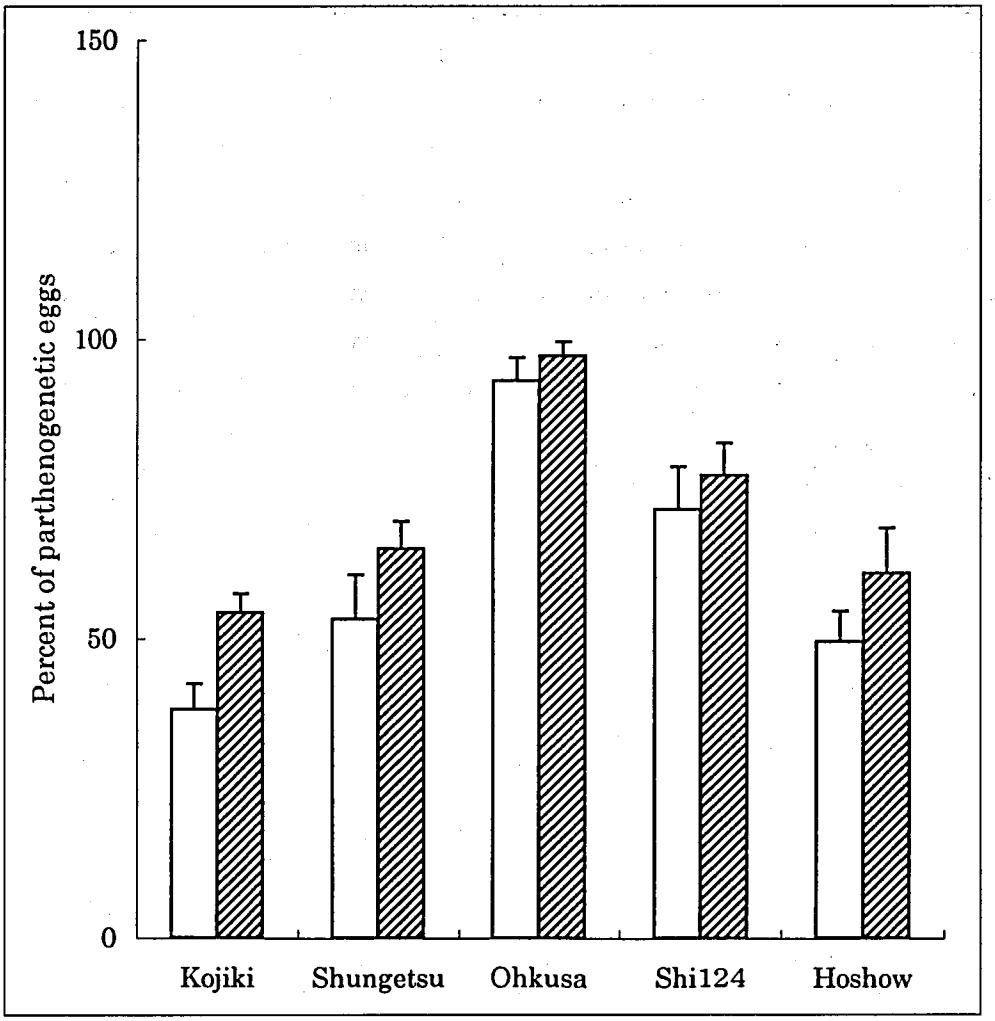

Fig.4. Racial difference in percent of parthenogenetic eggs treated with ecdysterone injected in a pupal stage. Open and hatched bars represent a non-injected and ecdysterone injected eggs. T-marks represent standard deviation.

Fig. 4.

The parthenogenetic rate in the case of ecdysterone-administration in all of the races was wholly reverse to the case of juvenile hormone and, even in the manifestation of parthenogenetic eggs, it was considered that antagonistic action was present between both hormones.

Ⅳ. Eelectron-Microscopic Observation of Egg by Administration of Endocrine Hormone: The change of the surface structure of egg accompanied by fertilization is a well known phenomenon. Hereupon, in order to investgate the relation of the difference between the manifestation states of parthenogenetic eggs by the administration of hormone to the change of the surface structure of eggs in this experiment, an electron microscope was used. When juvenile hormone was administered, a follicle cell became small and the amount of dark viscous substance in a cell was little as compared with the control group. Contrarily, in the case of the administration of ecdysterone, the follicle cell became large and the dark viscous substance was much confirmed. It is known that the follicle cell has function for incorporating protein in oocytes during growth from blood during the formation of eggs (Roth and Porter, 1964: Telfer, 1965). Hereupon, the follicle cell was observed in detail by the electron microscope. By the administration of juvenile hormone, the shape of a nucleus was relatively near to a spherical or oval shape in many cases. Further, the amounts of the mitochondria, granular

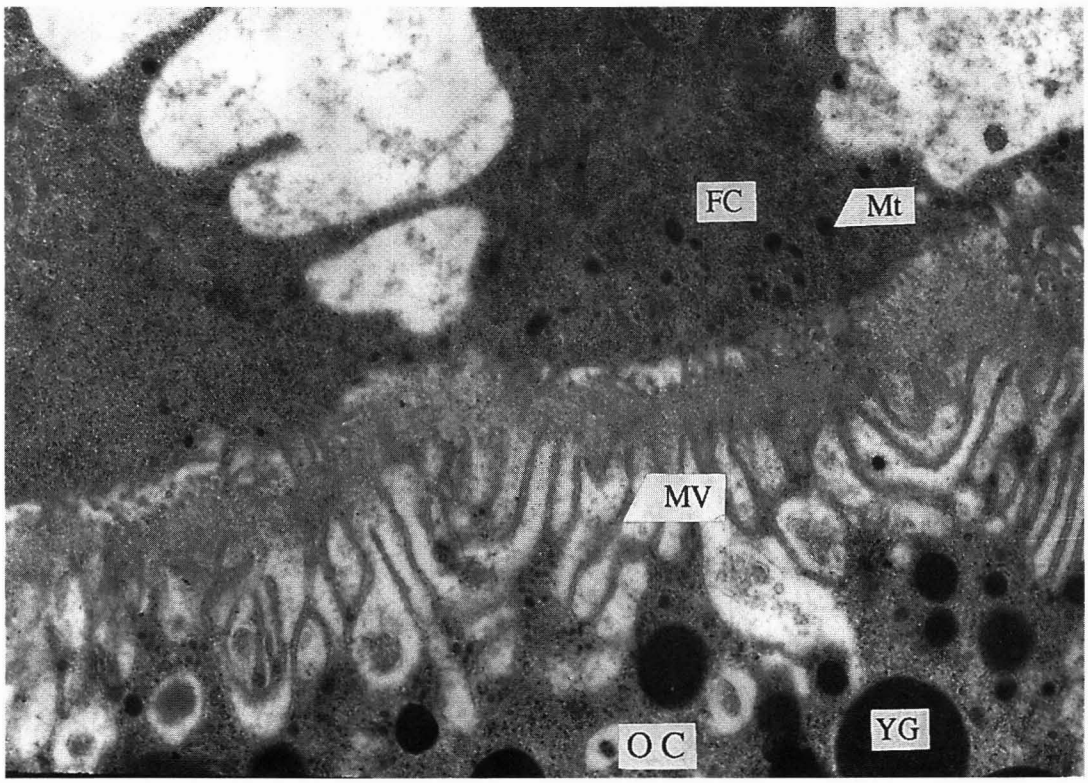

Fig. 5. Electron microscopic figures of by a non-endocrine hormone treatment (Control).
Footnote: FC: Follicle Cell. MV: Microvilli. Ly: Lysosome like substance. Mt: Mitochndrion. OC: Oocyte. YG: Yolk granule.

endoplasmic reticulum and fatty granule in a cell were little as compared with the control group, Contrarily, in the ecdysterone administration group, the nucleus was amorphous and intracellular granular endoplasmic reticulum was markedly formed. Therefore, it is considered that the synthesis of protein in the follicle cell is actively performed. Mitochondria was composed of dark viscous matrix and had an elongated shape in many cases and a large number of images proximate to division were also confirmed. Further, from such an aspect that mitochondria was mostly present in adjacent relation to fat droplets, it was suggested that the effective conversion of lipid was performed with respect to metabolism. In addition, many lysosome like granulus were observed.

At last, the comparative investigation was conducted on the forming state of a microvilli observed between the follicle cell and the oocyte (Fig. 5, 6, 7). The formation of the microvilli in the juvenile hormone administration group was inferior to that in the control group and granules taken in eggs were small and few. On the other hand, in the ecdysterone administration group, the microvilli was markedly formed and granules were also

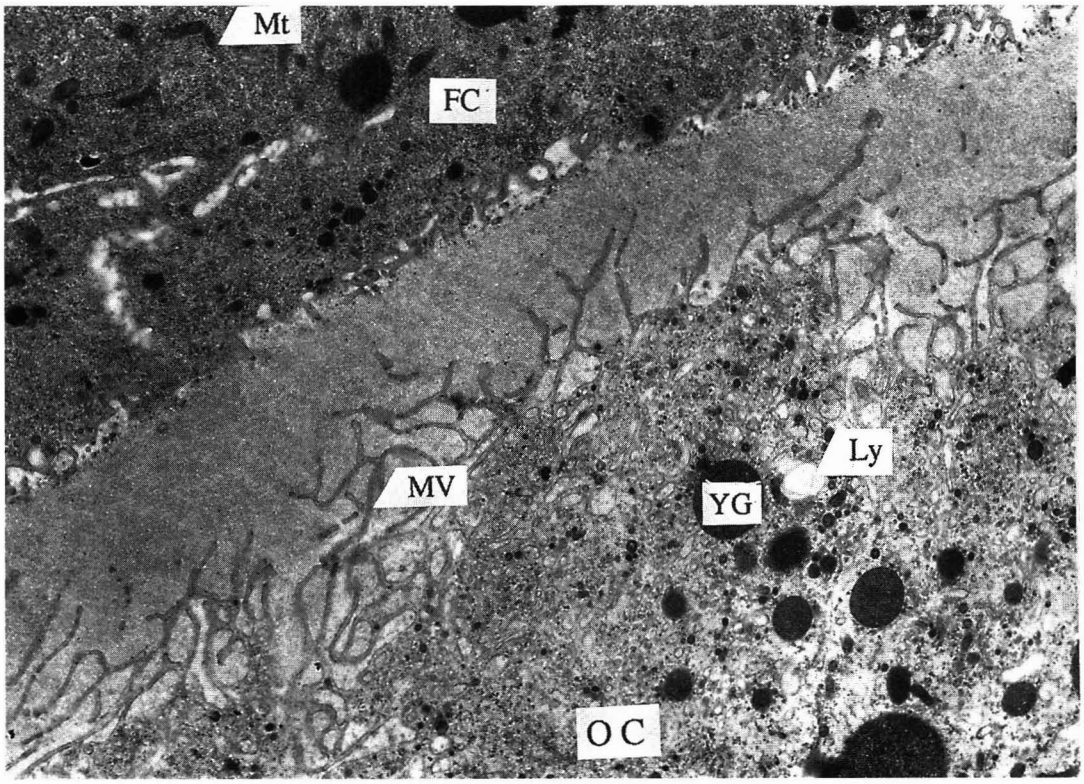

Fig.6. Electron microscopic figures of eggs by a juvenile hormone treatment. The formation was inferior in juvenile hormone treatments compared with control ones.

much. Therefore, it is considered that introduction of yolk protein into eggs is suppressed by juvenile hormone but promoted by ecdysterone.

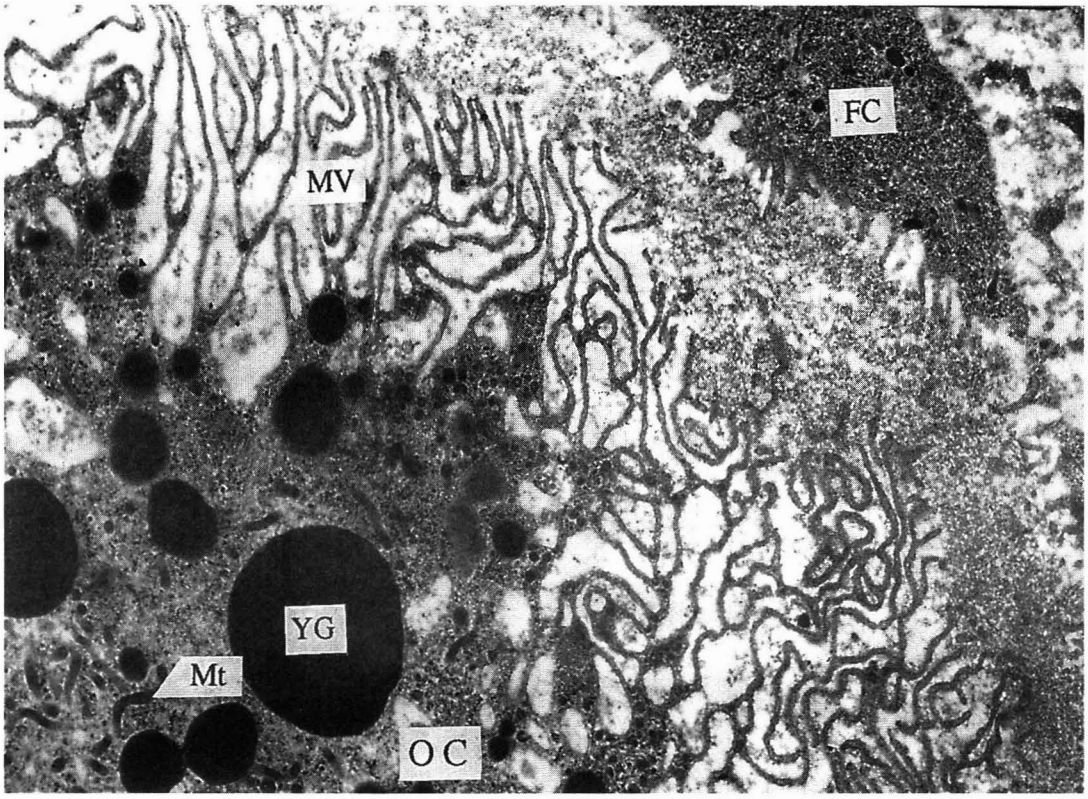

Fig.7. Electron microscopic figures of eggs by an ecdysteroid hormone treatments.
The formation of microvilli was more superior in ecdysteroid hormone treated than the others.

V. Electrophoretic Image of Intraovular protein by Administration of Endocrine Hormones: No large difference was confirmed between the electrophoretic image of the juvenile hormone administration group and that of the control group but a tendency to become little in the amount of female specific protein occurring from vitellogenin (electrophoretic band shown by the arrow in Fig. 8) was confirmed.

On the other hand, in the ecdysterone administration group, it was confirmed that the electrophoretic band showing a good migration characteristic was small as compared with the former two groups but, contrarily, female specific protein became much in quantity. From this, it is also considered that the protein occurring from vitellogenin has relation to the manifestation of parthenogenesis.

From above many aspects, it was considered that the degree of parthenogenetic rate was determined on the basis of the amount of intraovaular protein regulated by endocrine hormone. However, with respect to this problem, it will be necessary to conduct experiments in more detail prior to drawing a conclusion.

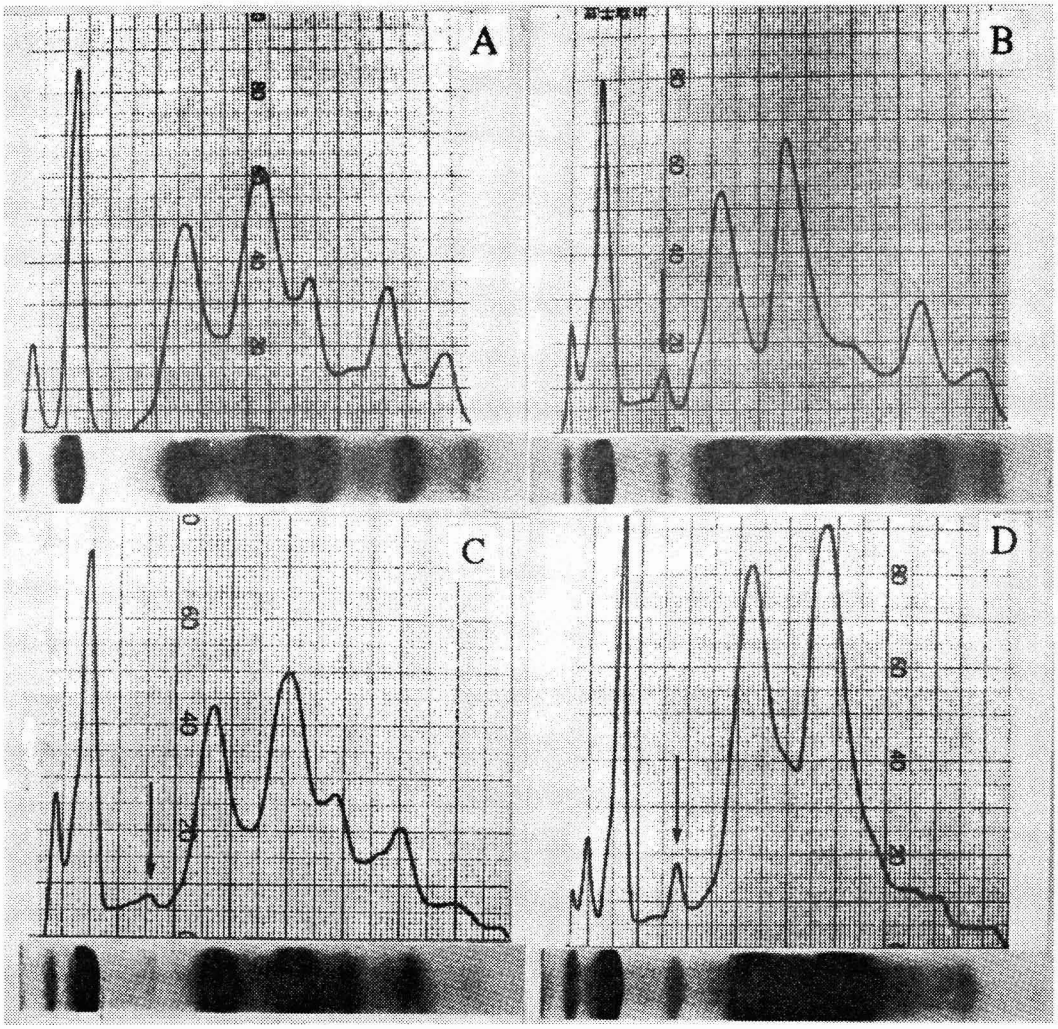

Fig.8. Electrophoretic pattern of egg protein in the silkworm.

A represent of male blood protein. B, C and D represent an e lectrophoretic pattern of eggs in case of non-endocrine hormone (control), juvenile and ecdysteroid hormone administration.

The electrophoretic band shown by the arrow Indicate a female specific protein.

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家蚕における単為発生に及ぼす内分泌ホルモン（幼若ホルモン・羽化ホルモン）の影響について

家蚕における単為発生の機構は、正常な卵子と精子の受精による個体発生と異なり、極体と卵子との受精による個体発生であることが明らかにされた（佐藤春太郎：1934)。また卵の発育状態と受精とは密接な関係にあることも知られている。そこで、内分泌ホルモンでもその作用機作を異にする二つのホルモン、すなわち、幼若ホルモン（Juvenile Hormone：いつまでも若い状態を保つ）と羽化ホルモンのエクジステロン（変態ホルモン）を使って、単為発生率、卵の組織変化および卵黄タンパク質などの変化を調べた。その結果次のようなことが判明した。

1）単為発生の発現率はエクジステロン処理が最も高く、次に無処理となり、幼若ホルモン処理のものが最低であった。この現象は原種と一代交雑種の双方で認められ、後者では特に顕著となり、雑種強勢（ヘテローシス）の現象が認められた。
2）卵の組織変化を電子顕微鏡で調べたところ、卵と包卵皮膜組織間に形成される微絨毛の形成状態はエクジステロン処理が最も顕著に形成されていたが幼若ホルモン処理では逆にその形成が最も抑制されていた。
3）卵内に貯留される雌特異タンパク質を電気泳動法で調べ、そのタンパク質量をデンシトメーターで測定した。その結果、そのタンパク質形成量についてもエクジステロン処理が最も多く、次いで無処理となり、幼若ホルモン処理のものは最少であった。

以上の結果より、単為発生の発現状況は内分泌ホルモンによって調節される卵内タンパク質量により決定されるものと考えられる。