Tissue Culture of the Rice Stem Borer, *Chilo suppressalis* Walker  
(Lepidoptera: Pyralidae)

I. Cell Migration from the Explanted Tissues of Diapausing Larvae

By Jun Mitsuhashi

Division of Entomology, National Institute of Agricultural Sciences, Tokyo

INTRODUCTION

Since Goldschmidt (1915) cultivated spermatocytes of *Hyalophora cecropia*, many investigators have tried to cultivate tissues of lepidopterous insects *in vitro*. Trager (1935) succeeded in cultivation of ovariole tissue of *Bombyx mori*, and cultivations of the ovariole tissues of lepidopterous insects were further developed by many other investigators (Wyatt, 1956; Grace, 1958 a, b, 1959; GAW, Liu and ZIA, 1959; Medvedeva, 1960; Jones and Cunningham, 1960, 1961; Hartzell, 1961). Recently, Grace (1962) established cell lines from the ovariole tissues of *Antheraea eucalypti*. Cultivations of other tissues have also been attempted (Wyatt, 1956; Loeb and Schneiderman, 1956; Loeb, 1957; Grace, 1958; GAW, Liu and ZIA, 1959; Medvedeva, 1960; Martignoni and Scallon, 1961; Sen Gupta, 1961, 1963; Hirumi and Maramorosch, 1964), but no better growth has been obtained than that of ovariole tissues. The present paper describes the results obtained from cultivations of various tissues of *Chilo suppressalis* Walker diapausing larvae.

MATERIALS AND METHODS

Insect material *C. suppressalis* larvae used in this study were reared on an artificial diet under aseptic conditions (Kamano, 1964). Diapause was artificially induced by keeping the larvae under short photoperiod at 25°C (Inoue and Kamano, 1957). Diapausing larvae were chosen as materials, since they have some advantages over non-diapausing larvae or pupae. *C. suppressalis* larvae enter diapause at their full grown state and the diapausing larvae are considered to be physiologically uniform. They can be stored for long periods at room temperature without supplying any diet.

Culture medium The medium consisted of 50 mg NaH$_2$PO$_4$·H$_2$O; 120 mg MgCl$_2$·6H$_2$O; 160 mg MgSO$_4$·7H$_2$O; 120 mg KCl; 40 mg CaCl$_2$·2H$_2$O; 80 mg D-glucose; 520 mg lactalbumin hydrolyzate; 520 mg Bacto-peptone; 40 mg choline chloride; 200 mg TC-yeastolate; 20 ml TC-199 medium; 20 ml fetal bovine serum; 5 mg streptomycin; and distilled water added to make 100 ml. The pH was adjusted to 6.2 with KOH. The fetal bovine serum and the TC-199 medium were purchased from Microbiological Associates, Inc., Bethesda, Md., U.S.A., the TC-yeastolate and the Bacto-peptone from Difco, Detroit, Mich., U. S. A., the lactalbumin hydrolyzate from Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A., and the dihydrostreptomycin sulfate from Takeda Chemical Industries Ltd., Osaka, Japan. The culture medium was sterilized by filtration. The ready to use medium could be kept in a refrigerator for several weeks without deleterious effects.

Culture vessel The same type of culture vessel as that used for leafhopper tissue culture was employed (Mitsuhashi and Maramorosch, 1964). The vessels consisted of microslide rings of 25 mm diameter and 10 mm height and cover glasses. After sterilization of the microslide rings and cover glasses by dry heat at 180°C for 2 hours, one side of the ring was closed with a cover glass by means of paraffin-balsam (1:1)

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just before setting up the culture. The opposite side was closed with another cover glass by means of grease after the culture was set up. Tissues were cultivated on the bottom of the culture vessel, so that observation was easily made by an inverted microscope.

**Technique** Since larvae were reared under aseptic conditions from eggs, sterilization of animals was not necessary. Organs or tissues were dissected out of diapausing larvae in sterilized RINALDINI's salt solution (RINALDINI, 1954). The dissected organs or tissues were placed on MAXIMOV slides in sterilized RINALDINI's solution and cut into small pieces. The tissue fragments were then transferred onto a MAXIMOV slide with a few drops of sterilized solution of 0.1 per cent trypsin (Trypsin 1:250, purchased from Difco) dissolved in RINALDINI's solution. Trypsinization was carried out at room temperature of approximately 25°C for 10 minutes. Trypsinized tissue fragments were transferred into a modified RINGER-TYRODE's salt solution (CARLSON, 1946) placed on the bottom of a culture vessel. Tissue fragments attached themselves to the glass surface almost instantaneously when they were released from a pipette into the modified RINGER-TYRODE's solution. The RINGER-TYRODE's solution was then replaced with the culture medium, which was done by carefully changing the medium several times. Finally the tops of the vessels were sealed with cover glasses by means of grease. All procedures were carried out under sterile conditions. Glassware was sterilized by dry heat at 180°C for 2 hours, and the dissecting dish and instruments were sterilized by submersion in 70 per cent ethyl alcohol. The salt solutions and trypsin were sterilized by filtration.

**Conditions of cultivation** During the cultivation, cultures were kept at 25°C. Light conditions were not controlled. The culture medium was changed once a week.

**Observation** The status of growing cells was examined with a Nikon MD inverted phase contrast microscope. Photomicrography and time-lapse cinematography for recording cell behavior were made with this microscope. For histological examination, cultures were preserved permanently by fixing with BOUIN's fluid and staining with DELAFIELD's haematoxylin-eosin. Various histochemical techniques were also applied when necessary.

**RESULTS**

**Nervous systems** From the explanted abdominal ganglion, wandering cells migrated within 24 hours after the culture was set up (Plate I, Fig. 1). These cells were quite mobile and wandered about by amoeboid movement. The cytoplasm of these cells contained granules of various sizes, and was distributed densely around nuclei. Thin cytoplasmic processes spread from these cells. The diameters of the cell and the nucleus were about 50 μ and 8 μ respectively. These cells were short-lived and some of them began to degenerate 5 days after the culture was set up. Large epithelial cells also appeared from the explanted abdominal ganglion (Plate I, Fig. 2). These cells did not form large cell sheets. They were very flat, and the cytoplasm was thin and contained small granules. Most of these cells were elliptical in shape and their major diameter was about 120 μ. The nucleus was round and its diameter was about 13 μ. These cells survived for a week. From the suboesophageal ganglion, very slender cells came out (Plate I, Fig. 3). The length of the cell was over 200 μ, while its width was only about 10 μ at its widest part. The diameter of the nucleus was about 8 μ. These cells did not leave the explanted tissues, and survived for about 10 days. No cell migration occurred from the brain, the frontal ganglion, and the complex of corpus cardiacum and corpus allatum.

**Circulatory organs** Explants consisted of fragments of hearts and alary muscles. Three types of cells were obtained from these explants. Cells of the first type were very flat epithelial cell-like cells. These cells formed loose cell sheets (Plate I, Fig. 4). The cytoplasm was very thin. Very
fine granules and small vacuoles were seen in the cytoplasm (Plate I, Fig. 5). The diameters of the cell and the nucleus were about 100 µ and 20 µ respectively. These cells survived for about 30 days. Cells of the second type were very slender and fiber-like (Plate I, Fig. 6). The length of the cells was over 250 µ and the greatest width was about 15 µ. They survived for about 30 days. Sometimes these cells developed pseudopodia-like cytoplasmic processes from their tips (Plate I, Fig. 7). Cells of the third type were very large and flat. They were spherical, elliptical or ovoid (Plate I, Fig. 8). The largest one measured 800 µ in diameter. Usually these cells were poly-nucleate. The nucleus sometimes took on a very long and slender form (100 µ long and 10 µ wide). The cytoplasm was very thin and often formed blisters. The cells survived for about 20 days. Explanted heart tissues maintained their pulsative movement for about 10 days.

**Alimentary canals** From the explanted midgut fragments, epithelial cells migrated. These cells formed cell sheets around the explants (Plate I, Fig. 9), but some cells wandered out of the cell sheets. The diameters of the cell and the nucleus were about 80 µ and 13 µ respectively. The cytoplasm of these cells contained granules of various sizes and vacuoles which were mostly distributed around the nuclei (Plate II, Fig. 10). The cytoplasm formed blisters. These cells resembled the type E epithelial cells obtained by cultivation of leafhopper midgut tissues (MITSUHASHI and MARAMOROSCH, 1964). These cells survived for about 15 days.

**Malpighian tubules** If the Malpighian tubules of diapausing larvae were subjected to trypsinization, they were soon destroyed. Furthermore, when they were placed in RINALDINI's salt solution which was free from Ca²⁺ and Mg²⁺, for about 30 minutes before placing in the culture medium they were also destroyed. If the tubules were brought directly into the culture medium, they survived for about 10 days, and epithelial cell-like cells migrated from them (Plate II, Fig. 11). The migrated cells survived for about 5 days.

**Fat bodies** The explanted fat body fragments liberated large flat cells (Plate II, Fig. 12). The cytoplasm of these cells contained lipid droplets of various sizes. The diameters of the cell and the nucleus were about 200 µ and 20 µ respectively. When there was abundant accumulation of lipid droplets in the cytoplasm, the nucleus appeared to be depressed (Plate II, Fig. 13). Small lipid droplets fused together and formed large droplets in the cytoplasm. These cells developed pseudopodia and moved out from the explants. They survived for about 15 days. When they degenerated, the whole area of the cytoplasm was filled with lipid droplets.

**Salivary glands** When salivary glands were cut anteriorly, salivary products came out immediately from the cut opening as a jelly-like substance, and the anterior parts of the glands shrunk. The tissues of salivary glands free from their products, were slightly trypsinized and brought into the culture medium. No cell migration was obtained from the explanted salivary gland tissues. The explanted tissues did not show contraction movements, and were gradually histolyzed.

**Silk glands** Cells migrated from the epithelium of the silk glands. These epithelial cells formed loose cell sheets. The diameters of the cell and the nucleus were about 50 µ and 13 µ respectively. The cytoplasm of these cells contained granules of various sizes and vacuoles which were mostly distributed around the nuclei (Plate II, Fig. 14). These cells survived for about 30 days.

**Prothoracic glands** No cells migrated from the explanted prothoracic glands, although the cells of the glands looked healthy for about 2 weeks in the culture medium.

**Testes** Trypsinization of testes was avoided, since the spermatocysts were fragile. Three types of cells were obtained from the explanted testis tissues. Cells of the first type were small epithelial cells migrated from the cluster of spermatocysts. These cells formed small cell sheets (Plate II, Fig. 15). These cells seemed to originate from cyst cells which surrounded the spermatocytes. The diameters of the cell and the nucleus
were about 20 μ and 12 μ respectively. Mitoses were occasionally observed in these cells (Plate II, Fig. 16). These cells survived for about 50 days. Spermatocytes were often found in and on these small epithelial cell sheets (Plate II, Fig. 15). Spermatocytes sometimes underwent mitoses (Plate II, Fig. 17). When the spermatocytes happened to attach themselves to the glass surface, some of them elongated by ejecting their cytoplasm (Plate III, Fig. 18). A similar phenomenon has reported in other lepidopterous insects by TAKAKUSU (1924). Cells of the second type were large phagocyte-like cells which came out from the cluster of spermatocysts (Plate III, Fig. 19). The diameters of the cell and the nucleus were about 100 μ and 15 μ respectively. The cytoplasm of these cells was concentrated around the nucleus and granules and vacuoles of various sizes were seen (Plate III, Fig. 20). These cells survived for about 20 days. This type of cell resembled the wandering cells which were obtained by the cultivation of B. mori testes (HIBBARD, 1935). Cells of the third type were very large epithelial cell-like cells migrated from the explanted testes. These cells seemed to originate from the peritoneal sheaths of the testes. The diameters of the cell and the nucleus were about 300 μ and 30 μ respectively. The cytoplasm of these cells spread thinly and showed some streaked structures and contained some very fine granules (Plate III, Fig. 21). Sometimes the cytoplasm formed blisters. They survived for about 60 days. Similar cells were also obtained from the ovariole sheath tissues of the same species.

During cultivation, some of the explanted spermatocysts developed into spermatids. At the beginning of the cultivation, all of the spermatocysts of the diapausing larvae were spherical. They enlarged to about 150 μ in diameter and then became pyri-form a few days after the culture was set up. Later, they were transformed into spermatids by further elongation (Plate III, Fig. 22). The tail of sperms were recognizable in them. Sometimes thin cells came out from the sheath of developed spermatids (Plate III, Fig. 23). Similar cell migration has been reported in the cultivation of B. mori testes (MEDVEDEVA, 1960).

The vas deferens did not produce cell migration, although SEN GUPTA (1961) reported out-growth of cells from isolated pieces of vas deferens in Galleria mellonella.

Ovaries Four types of cells were obtained from the explanted ovariole tissues. Cells of the first type were small epithelial cells migrating from the cut opening of the explanted testes within 24 hours after the culture was set up (Plate III, Fig. 24). The diameters of these types formed loose cell sheets. The diameters of the cell and the nucleus were about 25 μ and 12 μ respectively. The cytoplasm contained granules and vacuoles. Oocytes were often found in and on these small epithelial cell sheets (Plate III, Fig. 25). These cells were rather short-lived and degenerated a week after the culture was set up. Following the migration of the small epithelial cells, wandering cells and large epithelial cells came out from the explants. Cells of the second type were wandering cells. The cells of this type showed active amoeboid movement and were distributed at random away from the explant (Plate IV, Fig. 26). The cytoplasm of the cells was concentrated around the nuclei and had small granules and some vacuoles. The thin cytoplasmic membrane spread around the centrally condensed cytoplasm (Plate IV, Fig. 27). The diameter of the cell and the nucleus were about 70 μ and 12 μ respectively. Sometimes mitoses were seen in these cells (Plate IV, Fig. 28). During cultivation, very large vacuoles appeared in these cells (Plate IV, Fig. 29), and most of such cells became round. They survived for about 60 days. Cells of the third type were large epithelial cells. The cells of this type seemed to originate from ovariole sheath tissues. These cells formed compact cell sheets (Plate IV, Fig. 30). The cytoplasm of these cells was thin and contained fine granules. The diameters of the cell and the nucleus were about 70 μ and 20 μ respectively. Mitoses were occasionally observed in these cells (Plate IV, Fig. 31). These cells survived for about 90 days. Cells of the
fourth type were very large epithelial cell-like cells. This type of cell also appeared to originate from the explanted ovariole sheath tissues. The diameters of the cell and the nucleus were about 200 µ and 20 µ respectively. The cytoplasm of these cells was very thin and radially streaked structures were seen in the cytoplasm. Some granules were found along these streaked structures (Plate IV, Fig. 32). These cells formed cell sheets around the explants, but cells situated at the periphery of the cell sheets often migrated out. The cell left the cell sheets became round and degenerated later. The cells of this type survived for about 60 days. This type of cell resembled the third type cell obtained from the cultivation of testes of the same species.

Oviducts also produced some growing cells. From the cut opening of the oviducts very slender cells migrated. The length of the cell was about 150 µ and the diameter of the nucleus was about 10 µ (Plate IV, Fig. 33). These cells survived for about 20 days.

DISCUSSION

Tissues of *C. suppressalis* larvae were quite tolerant to trypsinization. AIZAWA and VAGO (1959) reported that trypsinization had deleterious effects on the tissues of lepidopterous insects. In the case of *C. suppressalis* larvae, however, trypsinization stimulated the ability of most of the tissues to produce migrating cells. The adhesiveness of the tissues to the glass surface was also increased by treatment with trypsin.

Of all the tissues used as explants, the gonads gave the best results. The wandering cells and the large epithelial cells obtained from the explanted ovariole tissues survived for long periods and multiplied by mitoses. These cells seem to be promising for subculturing and establishment of cell-lines. They seem to be identical with cells obtained in ovarian tissue culture of other lepidopterous insects (WYATT, 1956; GRACE, 1959; JONES and CUNNINGHAM, 1961; SEN GUPTA, 1961). The wandering cells are similar in shape to the plasmatocytes of the same species cultivated *in vitro* (MITSUHASHI, unpublished).

In experiments on polyhedral infections, SEN GUPTA (1963) cultivated the fat body cells of *G. mellonella*. Similar fat body cells migrated from the explanted larval fat bodies of *C. suppressalis*, but they did not multiply by mitoses and were not able to survive for long. If they could be multiplied or be kept alive for a considerable period, they may provide suitable materials for the study of virus multiplication *in vitro*, because fat bodies are known to be infected by various viruses.

During the cultivation of male gonads, spermatocysts developed into spermatids and sperms were formed in them. Since GOLDSCHMIDT (1915, 1916) observed spermatogenesis of *H. cecropia* in hanging drops of blood, the development of spermatocysts *in vitro* has been obtained using media containing insect hemolymph (LEWIS, 1916; LEWIS and ROBERTSON, 1916; GAW, LIU and ZIA, 1959; MEDVEDEVA, 1960). But there is no record of the *in vitro* development of spermatocysts in media without insect hemolymph. By the cultivation of male gonads in hanging drops of blood, SCHMIDT and WILLIAMS (1953) showed that spermatocysts of diapausing pupae of *H. cecropia* and *Samia walkeri* (cynthia) could develop into spermatids only when the blood contained a growth-promoting hormone probably secreted from the prothoracic glands. The culture medium used in this study contained neither insect hemolymph nor purified insect hormones. If a similar mechanism of spermatocyst development occurs in *C. suppressalis*, then it can be suggested that some substance in the chemically undefined components of the medium fulfils the role of the prothoracic gland hormone. Further studies on the hormonal regulation of spermatocyst development *in vitro* are under way.

SUMMARY

Tissues of *Chilo suppressalis* diapausing larvae were cultivated *in vitro*. Various tissues were dissected out of larvae under
aseptic conditions. Fragments of isolated tissues were then subjected to trypsinization, and cultivated in the synthetic medium by changing the medium once a week. From nervous systems, three types of cells were obtained. Heart and alary muscles also produced three types of migrating cells. Alimentary canals, Malpighian tubules, fat bodies, and silk glands each liberated one type of cell. Male and female gonads gave rise to three and four types of cells respectively. No cell migration occurred from explanted salivary glands and prothoracic glands. The wandering cells and the large epithelial cells obtained from the explanted ovariole tissues are the most promising for subculturing and establishment of cell-lines, because they were able to survive for long periods and multiply by mitoses. Spermatocysts developed into spermatids in a medium which contained neither insect hemolymph nor purified insect hormones.

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REFERENCES

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EXPLANATION OF PLATES

Plate I. Cell migration from the explanted tissues of Chilo suppressalis diapausing larvae. Bright phase contrast.

Fig. 1. Wandering cells migrated from abdominal ganglion. (× 600)
Fig. 2. Large epithelial cell migrated from abdominal ganglion. (× 600)
Fig. 3. Migration of slender cells from suboesophageal ganglion (Sg). (× 100)
Fig. 4. Loose epithelial cell sheet formed around explanted heart tissue (H). (× 100)
Fig. 5. High magnification of epithelial cells shown in Fig. 4. (× 470)
Fig. 6. Very slender cell obtained by the cultivation of heart tissue. (× 250)
Fig. 7. The same type of cell as shown in Fig. 6, with pseudopodia-like cytoplasmic processes. (× 250)
Fig. 8. Very large flat cell obtained by the
cultivation of heart tissue. The cell has three nuclei (N), and the cytoplasm form blisters (B). (× 100)

Fig. 9. Migration of epithelial cells from mid-gut tissue (M). (× 100)

Plate II. Cell migration from the explanted tissues of Chilo suppressalis diapausing larvae. Bright phase contrast.

Fig. 10. Epithelial cells migrated from midgut. (× 500)
Fig. 11. Cells migrated from Malpighian tubules. (× 800).
Fig. 12. Migration of fat body cells from the explanted fragment of fat body (F). (× 150)
Fig. 13. Migrated fat body cell with many lipid droplets. (× 600)
Fig. 14. Migration of cells from silk gland (Si. gl.). (× 180)
Fig. 15. Small epithelial cells migrated from the cluster of spermatocytes. Spermatocytes (Sc) are seen in and on the cell sheet. (× 430)
Fig. 16. Side view of mitosis (arrow) at metaphase observed in the small epithelial cell from the cluster of spermatocytes. Chromosomes are on an equatorial plane. (× 700)
Fig. 17. Side view of mitoses (arrows) at metaphase observed in spermatocytes which migrated out of cluster of spermatocytes (Scy). Chromosomes on equatorial plane are seen as short straight lines. (× 1,000)

Plate III. Cell migration from the explanted tissues of Chilo suppressalis diapausing larvae. Bright phase contrast.

Fig. 18. Elongated spermatocytes by ejecting their cytoplasm. (× 450)
Fig. 19. Phagocyte-like cells migrated from the cluster of spermatocytes. (× 100)

Fig. 20. High magnification of the cells shown in Fig. 19. (× 450)
Fig. 21. Very large epithelial cells migrated from peritoneal sheath of testis. (× 100)
Fig. 22. Spermatids (St) developed in the medium without insect hemolymph. Some undeveloped spermatocysts (Scy) are also seen. (× 100)
Fig. 23. Cell migration from the sheath of developed spermatids. (× 600)
Fig. 24. Migration of small epithelial cells from the cut openings of ovarioles (Ov). (× 150)
Fig. 25. Loose cell sheet of small epithelial cells. Some oocytes (Oc) are also seen in and on the cell sheet. (× 450)

Plate IV. Cell migration from the explanted tissues of Chilo suppressalis diapausing larvae. Bright phase contrast.

Fig. 26. Wandering cells migrated from ovariole. (× 100)
Fig. 27. High magnification of the cells shown in Fig. 26. (× 430)
Fig. 28. Side view of mitosis at metaphase observed in a wandering cell migrated from ovariole. Chromosomes (Ch) were on an equatorial plane. (× 800)
Fig. 29. Wandering cells with large vacuoles in ovariole culture. (× 430)
Fig. 30. Large epithelial cells migrated from ovariole (Ov). (× 150)
Fig. 31. Side view of mitosis at metaphase observed in a large epithelial cell. Chromosomes (Ch) are on an equatorial plane. (× 700)
Fig. 32. Very large epithelial cell with radially streaked cytoplasmic structures which migrated from ovariole. (× 400)
Fig. 33. Migration of slender cells from the cut opening of oviduct. (× 100)

摘 要
ニカメイチュウの組織培養

I. 休眠幼虫組織からの細胞移住

三 橋 淳
農林省農業技術研究所

ニカメイチュウの休眠幼虫の組織を in vitro で培養した。材料には人工飼料で無菌飼育し、短日条件によって人工的に休眠をとらせた幼虫を用いた。幼虫を滅菌した培地溶液中で溶解して、必要な器官または組織をとり出し、それを細切した後、軽くトリプシンで処理し、合成培地中で培養した。培地は無機塩、糖、ラクトアルプミン加水分解物、ペプトン、塩化コリン、酵母抽出物、TC-199培地、牛胎児血清、ストレプトマイシンからなり、pH は 6.2 に調整された。培養容器は 2 枚のカバーグラスとガラス製のスライドリングを組み合わせて作られた。とり出された幼虫の組織は培養容器の底面のカバーグラス表面で培養され、細胞の移住、増殖の状態は日
本光学ミク aime立顕微鏡を用いて観察された。腹部神経球から遊走細胞と大形上皮細胞がえられたが、いずれも短命であった。唾液下神経球からは細長い細胞が移住した。腸管および盲腸からなる移植片からは、上皮細胞、線維状細胞、大形の多核細胞がえられた。中腸から時には水状構造をもとめる上皮細胞状細胞が移住した。マレキー氏管および糸腺からは上皮細胞がえられた。脂肪体からは、油滴を多数含む脂肪細胞が移住した。精果からは大小2種の上皮細胞と遊走細胞がえられた。卵巣からは3種の上皮細胞と1種の遊走細胞がえられた。唾液腺、前腸腺からは細胞の移住増殖は起らなかった。これらの細胞のうち、精果由来の小形上皮細胞、卵巣由来の大形上皮細胞および遊走細胞は有余分裂を行なって増殖し、かなりの期間生存した。

一方、精果培養の際 in vitro で spermatocyst が発育して spermatid がえことができた。休眠幼虫では spermatocyst はすべて未熟で球形をしているが、培養を始めると、まず西洋梨型になり、さらに伸長して長い spermatid になり、その中に完成した個々の精子を認めることができた。一般に精子形成には前腸脇ホルモンが必要と考えられているので、この点は今後さらに検討する必要があると思われる。

新刊紹介

原色日本蛾類幼虫図鑑（上）
一色周知監修・六浦晃・山本義男・服部伊洛子共著（1965）、A5、237pp。原色60PL。保育社、1,800円
本上巻には日本産のスズメ蛾・ヤマユガ・イボタガ・カノコガ・コブガ・ヒトリガ・トラガ・ヤガ・ドクガの各科の幼虫193種が収録と共に、原色写真版によって図説されている。実際には近似種についての記載が本文中にあるから、約200種以上の中のある種が含まれている。保育社から既に刊行されている原色日本蛾類図鑑（上・下）には約3,000種が図説されているから、それに比較して決して多い種類ではないし、珍品と称するものも少ないかも知れない。しかし、われわれ農業害虫学にたずさわっているものにとっては、害虫の大部分を調べることができる。ただしこれも害虫としていないものであるから、実験を要しているなら、愛好者のみならず実用的価値は一層増やすだろう。

巣の幼虫や蛹の観察も簡便にして要を得いてためになる。応用昆虫学にたずさわる人々が手許におかれれば至便の書といえよう。

最後に、研究のための飼育など、著者らの苦心も容易でなかったであろうと、深く敬意を表すると共に、下巻を早く発行されるよう切望する。

（農技研・湯崎健）

抄

セクロピア蚕の変態期における DNA 合成
セクロピア蚕およびシング蚕のいろいろな変態時H^1^-チミジンを注射し、ミクロラジオオートグラフィーによりDNAへのチミジンのとりこびを追跡した。
中腸と後腸間の真皮細胞内に存在している酵母基は幼虫のステージが進むにつれて大きくなるが、ときに前収縮になると急激に成長し、さくらんじ核分裂をおこないDNAの合成をおこなうようになる。化蛹後2週間で25%をしめる内皮が形成され、真皮細胞や他多形の組織のDNAへのチミジンのとりこみがさかんにもみられる。化蛹2週間を過ぎると重量変化がいじるものに低下し休眠に入る。それにもとまない、酵母基、真皮細胞等でのDNA合成は全くとなり、精原細胞、精果の外皮細胞、血球でのわずかなDNAの合成が休眠期間中つくれられる。3ヶ月間の冷凍後25℃にて加温を開始すると、形態的な羽化への発育が観察される1週間前に、まず中腸の再生細胞、ついてて真皮細胞、酵母基、胸部節間筋肉、気管基、中腸神経細胞等でDNAの合成が再開される。脂肪腺内では形態的な変化がみられるようになってはじめてDNAの合成が始める。
なお休眠組織を備え、その後H^1^-チミジンを注射すると、とくに血球でのDNA合成がさかんになり、肝臓の節原細胞や中腸の再生細胞もチミジンのDNAへのとりこみが観察されるようになる。死後の遺伝子のとりこみを観察するとDNA合成がみられず、他組織間筋肉、酵母基も傷たたるものやDNA合成が見られない。これらの組織では休眠より覚醒することによって初めてDNAの合成が再開されることから、これらの細胞ではエクサソンが分泌されたときのみDNA合成が刺激されることが推定されている。

（東大農・藤塚勢夫）