Appearance of Iridescence in the Tissues of the Rice Stem Borer Larvae, Chilo suppressalis Walker, Infected with Chilo Iridescent Virus (Lepidoptera : Pyralidae)

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Infection of various tissues of Chilo suppressalis larvae with Chilo iridescent virus (CIV) was examined by the appearance of iridescence. The CIV infected larvae were obtained by intrahemocoelic inoculation of CIV suspension under aseptic conditions. The inoculated larvae usually began to turn purplish 5 days after inoculation at 25°C. Pericardial cells and suboesophageal gland cells showed iridescence at first and the iridescence was most prominent. The iridescence could be seen in these cells as early as 3 days following inoculation. Fat bodies showed the next prominent iridescence. Fat body lobules underneath the epidermis showed iridescence 3 days after inoculation, but the large lobes distributed around alimentary canal showed iridescence much later and the iridescence was not prominent. Silk glands and salivary glands showed iridescence on some parts of the glands at 4 days following inoculation, but the iridescence appeared only on the surface of the gland. Hemocytes began to show iridescence 5 days after inoculation. In the larvae of advanced infection, hemolymph was turbid. Epidermal cells showed prominent iridescence 6 days after inoculation. Alimentary canals and Malpighian tubules began to show iridescence at 5 days following inoculation, but the iridescence seemed to appear only on the surface of them. In the prothoracic glands, iridescence appeared only in the posterior regions of the glands. At an advanced stage of infection, tracheal epithelium showed iridescence. Nervous system, gonads, and muscles did not show iridescence.

INTRODUCTION

The first iridescent virus of insect was described by Xeros (1954) in Tipula paludosa, and was named Tipula iridescent virus (TIV), Steinhaus and Leutenegger (1963) reported the second iridescent virus from Sericesthis pruinosa, and named it Sericesthis iridescent virus (SIV). Both of TIV and SIV have been used as materials for various studies because of their large sizes, good yields and iridescent properties. The third iridescent virus was briefly reported from Aedes taeniorynchus and was named mosquito iridescent virus (MIV) (Clark et al., 1965). Very recently, the fourth iridescent virus was found in the rice stem borer, Chilo suppressalis Walker, and was named Chilo iridescent virus (CIV) (Fukaya and Nasu, 1966). This new virus resembles to both of TIV and SIV in many respects, but is evidently different from them.
Iridescence in the Tissues of CIV Infected *Chilo suppressalis*

In the present paper, infection of various tissues of *C. suppressalis* larvae with CIV was examined according to the appearance of iridescence which has been considered to be produced by the microcrystalline arrangement of CIV particles (WILLIAMS and SMITH, 1957; KLUG et al., 1959; MERCER and DAY, 1965). The effects of CIV on the growth and development of *C. suppressalis* will be reported elsewhere.

**MATERIALS AND METHODS**

The larvae of *C. suppressalis* were reared on a synthetic diet under aseptic conditions. The larvae which were artificially induced to diapause by short photoperiod treatment were used as experimental animals. CIV was obtained from whole homogenate of the diseased larvae by differential centrifugation. CIV suspension in RINGER-TYRODE'S salt solution (pH 7.0) was centrifuged at 4,000 r.p.m. for 30 minutes just before inoculation in order to avoid possible bacterial contamination.

Intrahemocoelic inoculation was made with fine glass capillary under ether anesthesia, and the wound produced by the injecting capillary was ligated with cotton thread to prevent bleeding after recovery from anesthesia.

In most cases, aseptic inoculation of larvae with CIV was carried out. In this case, bacterium- or fungus-free CIV preparation was obtained in the following manner. A diseased larva, which was infected only by CIV and not by any bacterium or fungus, was surface sterilized by submersion in 0.1 per cent mercuric chloride for 5 minutes, then dissected in sterile RINGER-TYRODE's solution. Fat bodies and dorsal vessels which showed iridescence were taken out, care being taken not to damage the alimentary canal. The fat bodies and dorsal vessels were homogenized with small amount of the sterile RINGER-TYRODE's solution, and centrifuged at 4,000 r.p.m. for 30 minutes. The supernatant was injected into the hemocoel of aseptically reared larvae by means of sterile glass capillary. These procedures were carried out under aseptic conditions. In this way aseptic larvae infected only by CIV were obtained with ease.

The injected larvae were kept at 25°C in petri dishes containing moistened filter paper or in sterile Erlenmeyer flasks containing sterile diets in the case of aseptic inoculation.

Oral administration of CIV was also attempted by adding the bacterium- and fungus-free CIV preparation into the sterile diets on which the 3rd instar larvae were feeding.

After inoculation daily examination was carried out on the inoculated larvae by dissecting 5 larvae at a time. Since iridescence was recognized only in reflected light, observation was made with low power dissecting microscope or with Leitz Pan Photo equipped with ultropak.

**RESULTS**

The larvae inoculated with CIV intrahemocoelically became infected without exception, while the larvae fed on the CIV-containing diets became infected with low percentage. No difference was observed in the mode of infection between
orally and intrahemocoelically infected larvae, but the orally infected larvae took much longer time to be infected. Hereafter the results obtained on the larvae inoculated intrahemocoelically will be described.

The sign of the CIV infection was recognized externally after 5 days. The larvae inoculated with CIV turned slightly purple at 5 days following inoculation, and the color became more and more purplish as the disease progressed. When larvae were inoculated under non-aseptic conditions, many larvae died on account of secondary bacterial infections soon after inoculation, while all the larvae survived for more than one month in the case of aseptic inoculations. After one month the infected larvae varied in color, from pale purple to deep purple.

Before the appearance of external sign of the CIV infection, some internal organs showed iridescence, indicating the multiplication of the virus.

_Circulatory organs_: Pericardial cells began to show iridescence 3 days after inoculation. Most cells were bluish purple and some were greenish purple or pink (Plate I, A). Dorsal vessels and alary muscles did not show iridescence. The circulatory organ was usually transparent and hardly recognized when dissected, but in the CIV infected larvae it was easily recognized, because it became bluish purple as a whole. The bluish tint turned deeper and deeper with the progress of infection. Small glittering greenish or bluish particles appeared in the pericardial cells after 5 days following inoculation. In the larvae of more advanced infection, the pericardial cells became swollen and in some larvae they became swollen and dark brown with iridescence. Even in such heavily infected larvae, the pulsative movement of the dorsal vessels were maintained.

_Suboesophageal glands_: The cells of suboesophageal gland began to show iridescence 3 days after inoculation. The pathological change of the cells were the same as that of the pericardial cells (Plate I, B). It was not easy to distinguish suboesophageal glands from the surrounding fat bodies in the normal larvae, while it became easy in the CIV infected larvae, because the glands as a whole became more purplish than the surrounding fat bodies.

_Fat bodies_: Fat body lobules underneath the epidermis showed iridescence 3 days after inoculation, while large lobes of fat bodies which were located around alimentary canal did not show iridescence at this time. The color of the lobules was bluish purple, greenish purple, orange or pink, and the cells contained glittering greenish or bluish particles (Plate I, C). The large lobes of the fat bodies began to show iridescence 7 days after inoculation, and the cells contained glittering greenish or bluish particles at this time. But the large lobes did not turn more bluish than the lobules underneath the epidermis.

_Silk glands_: The gland as a whole did not show marked changes. After 4 days, some parts of the gland, mostly middle and posterior parts, showed iridescence. The iridescence was, however, due to the thin layer of the cells which adhered to the gland, but not due to the gland cells themselves. These adhered cells were deep bluish purple and contained glittering bluish or greenish particles (Plate I, D). In some cases, some parts of the gland were swollen, and the surface of these parts showed iridescence.

_Salivary glands_: Some parts of salivary glands became swollen as nodes and the surface of these nodes produced iridescence 4 days after inoculation. But the iridescence did not appear from the inside of the glands, suggesting that the
Iridescence was produced by some other tissues adhered to the gland. The parts showing iridescence also revealed glittering greenish particles. 

**Blood**: Hemocytes began to show iridescence 5 days after inoculation. Most of the hemocytes became bluish purple and some became greenish purple or orange (Plate I, E). The infected hemocytes were spherical and swollen as compared normal hemocytes. Hemocytes of *C. suppressalis* consisted of 7 types of cells (Mitsuhashi, 1966), but in the CIV infected larvae these types of the hemocytes could not be distinguished. Usually some types of hemocytes developed pseudopodia when they were drawn out of hemocoel, but the hemocytes of the CIV infected larvae rarely developed pseudopodia. In the larvae at an advanced stage of disease, hemolymph became turbid and were found to contain numerous small particles showing iridescence. These particles were assumed to be derived from the destroyed cells by infection.

**Epidermal cells**: Two days after inoculation the epidermal cells became faintly bluish, and the iridescence turned prominent at 6 days following inoculation. At this time epidermal cell layer was deep bluish purple or yellowish green as a whole, and the cells contained glittering greenish particles (Plate I, F).

**Alimentary canals**: Some parts of mid-gut became faintly greenish purple 5 days after inoculation. Fore-gut and hind-gut also became bluish somewhat later (Plate I, G). The iridescence was seen only on the surface of the alimentary canal, and it was not sure whether the iridescence was produced by the epithelial cells of the alimentary canal themselves or by some other tissues such as connective tissues or fat bodies which adhered to the alimentary canal. The parts that turned purplish also revealed glittering greenish particles. Similar result was obtained in the case of oral infection.

**Malpighian tubules**: Some parts, mostly posterior parts, of the Malpighian tubules showed iridescence 5 days after inoculation. In the larvae of advanced infection, the tubules became faintly bluish as a whole, and small glittering bluish or greenish particles appeared in the tubules. But intensive iridescence was limited on the surface of the tubules.

**Tracheae**: Tracheal epithelium became bluish in late stage of the disease. The cells showing iridescence contained glittering greenish particles (Plate I, H).

**Prothoracic glands**: Iridescence appeared only in the posterior parts of the glands, at which the gland received tracheal supply, 5 days after inoculation. The parts became greenish blue and glittering greenish particles were seen in them. Near by the anterior branches of the gland, there was a particular large cell which always showed iridescence. But this iridescence was not produced by CIV, and the identical cell showed iridescence even in the non-infected larvae. This iridescence was very similar to the auto-iridescence of the neurosecretory cells of the brain, and supposed to have some relation to the hormonal activity.

**Other organs**: Nervous system, gonads of either males or females, and muscles did not show iridescence even in the heavily infected larvae. Gonads were always surrounded by the heavily infected fat body cells but gonads themselves did not produce iridescence.

**DISCUSSION**

The production of iridescence in the infected tissues is remarkable property of
the viruses of this group. These viruses form crystals spontaneously, either in vivo or in vitro, and the iridescence is produced by a Bragg-type diffraction arising from sets of planes of the crystalline lattice formed by the virus particles (Williams and Smith, 1957; Klug et al., 1959). The iridescence is, therefore, used as markers by which the virus can be unmistakably recognized in vivo. However, the absence of the iridescence does not necessarily mean the absence of the virus.

The larvae of C. suppressalis infected with CIV became purplish. This change of color mainly due to the appearance of iridescence in the epidermal cells. The iridescence produced by the infected pericardial cells and the fat body lobules underneath the epidermis also contributed to the external color change. The purplish coloration of the infected insects is the general property of the viruses of this group (Smith, 1958; Smith et al., 1960; Day and Mercer, 1964), and this facilitates the diagnosis of the infection due to these viruses.

Of all the internal organs, the pericardial cells and the suboesophageal gland showed the most marked changes. Iridescence appeared in these organs at first and was most prominent in these organs. The fact that pericardial cells and suboesophageal gland cells showed the same pathological changes seems to be reasonable taking into account the functions of these cells. Although the functions of the pericardial cells and suboesophageal gland cells have not been thoroughly investigated, it is certain that both cells do active phagocytosis. It is, therefore, probable that the selective appearance of iridescence in these cells at the early stage of the infection is due to the intake of many virus particles by these cells. Day (1965) reported the presence of SIV in the heart of the infected adult of Galleria mellonella by the Feulgen technique, but the detail was not reported.

The fat bodies have been thought as the location in which the iridescent virus mainly multiplied (Williams and Smith, 1957; Smith, 1958; Smith et al., 1961; Day, 1965; Clark et al., 1965), but the iridescence produced by the fat bodies infected with CIV was not marked as that of the infected pericardial cells and suboesophageal glands. Furthermore, it is noteworthy that the iridescence produced by the fat bodies was less prominent in main lobes which occupied considerable parts of hemocoel as compared with small lobules underneath the epidermis.

The epidermal cells showed bluish iridescence when infected with CIV. The presence of large amount of viral DNA in the epidermal cells has been demonstrated when G. mellonella was infected with SIV (Day, 1965). TIV has also been reported to be present in hypodermis of Tipula paludosa (Xeros, 1964), in epidermis of Lymantria dispar (Smith et al., 1961), and in skin of Mycetophila sp. (Smith et al., 1961), when larvae of these insects were infected with TIV.

The hemocytes have been known to be infected by the iridescent virus (Reutenegger, 1964; Day, 1965), but the relationship between cell types and viral infection has not been clarified. In the hemocytes of C. suppressalis infected with CIV, iridescence was different in color in different cells, and this suggests that there may be a slight variation in the mode of packing in the microcrystals, according to the cell types.

The tracheal epithelium of the larvae of C. suppressalis showed iridescence when
infected with CIV, and the same was true when G. mellonella was infected with SIV (DAY, 1965).

The iridescence has never appeared from muscles and nervous system of the larvae of C. suppressalis even if they were heavily infected with CIV, whereas SIV has been detected in the sarcoplasm of muscle cells, in the nerve cell bodies, and in the cells of the brain and of the nerve cord, when G. mellonella was infected with SIV (DAY, 1965). TIV also reported to be present in muscles of T. paludosa (XEROS, 1964), and of L. dispar (SMITH et al., 1961), when these larvae were infected with TIV.

Reproductive system of C. suppressalis did not show iridescence either in males or females infected with CIV. DAY (1965) reported that the viral DNA was not observed in reproductive system of G. mellonella infected with SIV, and that the virus could not be recovered from the progenies of the infected adults suggesting the absence of the transovarial infection of SIV in this species.

The iridescence observed on the gut of C. suppressalis infected with CIV was not sure to be produced by the epithelial cells of the gut or by some other tissues adhered to the surface of the gut. In G. mellonella infected with SIV, no virus was seen in cells of gut epithelium (DAY, 1965).

The diapausing larvae of C. suppressalis infected with CIV survived more than one month, in the longest case more than three months. Many larvae, however, died owing to the secondary bacterial infection shortly after the intrahemocoelic inoculation of CIV, unless bacterium-free virus preparation was inoculated aseptically reared larvae under aseptic conditions. On the other hand, the death due to the bacterial infection was rather rare when the non-sterile physiological saline was injected into larvae. It seems probable that the larvae infected with CIV lose their resistance to bacterial infection.

The multiplication of SIV has been studied in the GRACE’s insect cell line (BELLETT and MERBER, 1964; BELLETT, 1965 a, b). The GRACE’s cell line was said to be susceptible for SIV, but Antheraea eucalypti, from which the cell line was established, is not a natural host of SIV. On the other hand, C. suppressalis is a natural host of CIV and its tissues, especially hemocytes, can be maintained or cultivated in vitro for a considerable period in a synthetic medium (MITSUSHI, 1965, 1966). Now, the infection of various tissues of C. suppressalis larvae with CIV in vitro is underway.

REFERENCES


**EXPLANATION OF PLATE**

Plate I.

Iridescence appeared from various tissues of *Chilo suppressalis* larvae infected with CIV. Photomicrographs were taken on non-fixed and non-stained materials with Leitz Pan Photo equipped with ultropak. Sakura color negative 100 was used.

A: A part of circulatory system. D, dorsal vessel; F, fat body lobules. (×25).


C: Fat body lobules. T, trachea. (×30).

D: A part of silk gland. M, middle parts; P, posterior parts. (×30).

E: Hemocytes. (×150).


G: A part of mid-gut. T, trachea. Arrow indicates the cells showing iridescence. (×30).

H: Trachea. Arrow shows the tracheal epithelium showing iridescence. (×30).
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