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Studies on Nuclear Polyhedrosis Virus of Tussah Silkworm, *Antheraea Pernyi Guerin*

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摘 要

1. 韓國에서 柞蠶膿病을 發病시키는 主된 Virus는 核質多角體 Virus이다.

2. Virus 묶음은 封入體蛋白質의 分子構造內에 含入되어있다.

3. 封入體蛋白質에 存在하는 Virus 묶음은 平均 4개의 桿狀形 V rus粒子로 되어있으며 이를 싸고있는 막은 두개로 되어있다.

4. 封入體蛋白質內에 存在하는 Virus 묶음은 질서와 균형있게 배열된것이 아니고 흐는데로 散在해 있는것 같다.

5. Virus粒子와 封入體蛋白質은 傳染된 細胞의 染色體에서 형성된 소위 "Virogenic stroma"에서 생겨진것이다.

Many a fine structures of nuclear polyhedrosis virus in Lepidoptera had been described by electron microscope. In the larva of *Antheraea pernyi Guerin*, the leading virus causing infectious disease in Korea is disclosed nuclear polyhedrosis virus, which embed bundles of virus particles in the molecular lattice of polyhedra protein. The number of virus particles within a bundle is on the average four particles, which are enclosed in a intimate membrane closely surrounded with developi ng membrane. The bundles of four virus particlesare at random embedded in the polyhedra protein, which is originated from the so-called virogenic stroma of chromosom in the infected nuclear.

Introduction

The structure of nuclear polyhedrosis virus and granulosis virus in Lepidoptera was first investigated by electron microscopic examinations (Morgan et al, 1955, 1956; Day et al, 1956). The investigations disclosed that the virus particles are rod-shaped, surrounded by two membranes; one is named the intimate membrane, and the other the common developing membrane(Bergold, 1963). Other investigations(Hughes, 1953; Bergold, 1953) showed that the virus particles are in a ran dom position and randomly located within the molecular lattice of inclusion-body proteins.The virus do not play critical role in cristalization of inclusion body lattice and disturbe the formation of them(Bergold, 1963).

The process of virus replication in insects was revealed in detail by eletron microscopic examination of the ultra-thin sections(Harrap, 1969). These investigation showed that the virus particles approach to the susceptible cells through micro-villi after the nuclear polyhedrosis were dissolved in digestive juice of insect. Examination of micro-villi at the apex of some columner cells showed that occasional envelop virus particles were present(Harrap, 1969).

The purpose of the studies presently reported was to examine the fine structure of the virus particles when still enclosed within their inclusion bodies, and the developing process of them in the cells.

Material and Method

The purified polyhedra and the tissues of infected larva were fixed in 2 per cent osmium tetroxide(made up in trager's B) in fume cupboard for 1 hour and 30 minutes. The sample dehydrated in graded ethanols,

and finally transferred into gelatin capsules containing a drop of epikote resin to be polymerized overnight at 60°C.

Sections about 700~800Å thick were cut with a Huxley ultramicrotom equiped with a glass knife. The sections were placed on fenestrated formvar films on copper grids, and purified virus particles suspended in small droplets were applied with a pipete directly on the formvar, and stained with negative staining accordant to Brenner and Horne(1956) with phosphotungistic acid at pH. 7.0~7.5 by dropping the dye on-to the formvar grids. Most of electron micrographs were taken with an AEI EM 6 electron microscope at 50kv. using double condenser illumination, at initial magnification of 15,000~40,000.

Result

Several electron micrographs of sections through virus particles contained within polyhedra confirmed the morphological similar to that of silkworm, *Bombyx mori*(Linnaeus) (plate 1). The histopathology was also essencially similar to those of other Lepidoptera insects except for a few possible differences in the formation of virus particles which are described below.

Plate 1. *Antheraea pernyi*. Section of a purified polyhedron from the infected larva. Notice the densely stained virus particles within bundle and periphery of the polyhedron.

All virus particles appeared to be rod-shaped and surrounded by the developing membrane, but inthese electron micrograph it can hardely to discern the intimate membrane from the developmental membrane (Plate 2). The major part of the polyhedrosis virus particles contained within one bundles was 4, but some of them occured doubly (Plate 2).

Plate 2. *Antheraea pernyi*. Sections of bundles embedded in the polyhedron. Notice the four virus particles or two in a bundle.

Bundles of such numbers of particles that do not permit a symetric patterns were accordingly irregular (Plate 1, 2). An empty cavity was rarely found within a virus bundle (Plate 2) which was apparently the result of a widening the gap between the developmental and intimate membrane, although to some extent this may appear to be an artifact. Lonitudinal sections through virus rods revealed a dense and homogenous mass

Nuclear polyhedrosis virus infection of fat body tissu was observed from 120 hours to 216 hours after infection feeding the larvae of *A. pernyi Guerin*. The majority of the cells were usually virus-infected but the stage of development of the virus varied from cell. An early sign of virus infection was the enlargement of the nucleus and the formation of a large network of densely stained material often in a less well stained meshwork (Plate 3).

Plate 3. *A. pernyi.* The fat body cell showing virus development in the infected nucleus. Notice the clumpping nucleus, "virogenic stroma," developing virus particles and polyhedra.

Initially this network filled the nucleus almost entirely but rod-shaped virvs particles could be detected in association with it in the cells at later stage of infection and from a comparison various cells it was deduced that the network contracted as virus particles were produced (Plate 3). The network was called virogenic stroma by Xeros (1956) in order to distingish it from normal chromain. The size of the virogenic stroma appeared to decrease as large numbers of virus rods were formed in the nucleus. The areas of polyhedron protein appeared to grow around the virus rods that have become enclosed in outer membrane (Plate 4). During this process, the virogenic stroma disappeared as more virus rods and their seperate outer membrane were formed.

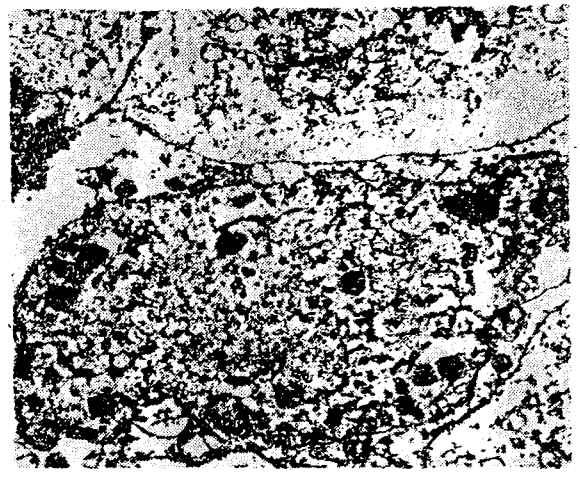

Plate 4. *A.hernyi.* Note the enveloped virus particles or empty developing membrane and maturing polyhedron.

The immature polyhedra increased in size, incorporatings larger numbers of virus rods until mature polyherawere formed (Plate 5). No enclosing membrane was observed around the polyhdera in both early and late stage of formation.

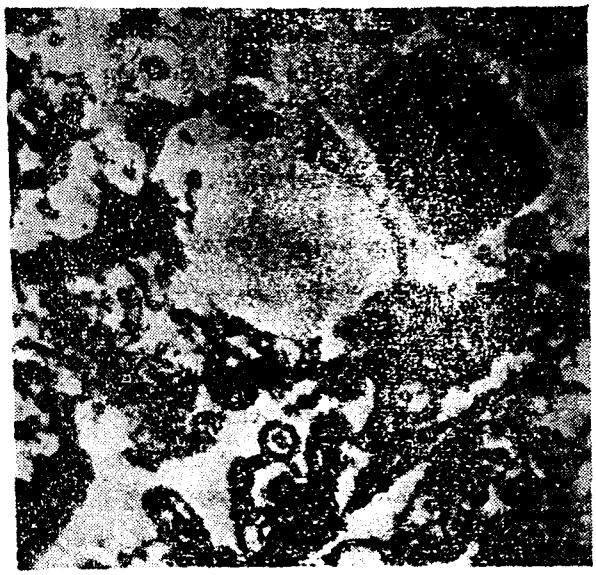

Plate 5. *A. pernyi.* The production of virus particles from the virogenic stroma and envelopment of virus particles. Note the possible formation of empty virus membrane.

Discussion

The number of virus particles within an intimate membrane in Lepidoptera varied from one to scores, but in the case of *A. Pernyi*(G.) it is limitted to four or two. No explanation can be, however, given as to why and by which mechanism different numbers of virus particles are included within one bundle.

The swelling of the virus envelope is probably a result of the passage of water and salts in solution across the virus envelope into the region between it and the virus particles.

The infected nuclues start to be round-off, and it contains large amounts of membraneus material, much of which is probably utilized in the development of the virus particles. Small massed of polyhedron protein were also present in all the infected nuclei examined and these were easily recognized by the presence of a pattern of the usual dimension. Enveloped virus particles were frequently observed on the periphery of much masses but no virus particles were surrounding an individual mass of polyhedron protein.

Though it was failed to make out the electron micr-

ograph for the approval, virus particles could be found within the microvilli through electron microscopic examinations, and in this situation surrounding virus envelope was no visible. It is therefor quite possible that function of the virus envelope is an important feature in the virus of gaining entry to the cell, if it is accepted that the virus particles seen in this situation around the microvilli were involved in the cell infection.

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