Characteristics of Densonucleosis in the Silkworm, *Bombyx* mori

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In 1968 a silkworm disease was prevalently epizootic in sericultural farms around the suburbs of Ina City, Nagano Prefecture, resulting in a great economic damage. The disease was supposed to be caused by an infectious flacherie virus (IFV) because of the similarity in symptom of the infected larvae. The virus isolated from the diseased silkworm was tentatively designated as "Ina isolate" of IFV and stocked in Nagano Sericultural Experiment Station. In 1974, however, Shimizu[1] found from the histopathology of the infected larvae with "Ina-isolate virus" that the virus was not an IFV, but a virus being formerly undescribed in the silkworm.

Since then, several investigators have studied in detail on the characterization of the disease and the virus. As the results, they concluded in common that the virus is different from IFV, but quite similar in cytopathological, physical, and chemical natures to a densonucleosis virus as known in the greater wax moth, *Galleria mellonella*. They also proposed that the disease should be named as *Bombyx* densonucleosis instead of infectious flacherie.

The major objectives of this article are to review the results of studies obtained so far on *Bombyx* densonucleosis.

General characteristics and histopathology of the disease

When the silkworm larvae are perorally infected with *Bombyx* densonucleosis virus (DNV), they usually die after seven days showing the body flacidity as a major symptom. On dissection, alimentary canal of the diseased larva shows pale yellow in color without most of the content. This sign is quite similar to that in the case of IFV infection. However, histopathological study[2] on the midgut epithelium of the diseased larva infected with DNV revealed that, although goblet cells were relatively intact, degraded columnar cells with hypertrophied nuclei were discharging from the epithelium into the gut lumen (Plate 1). This histopathological fea-

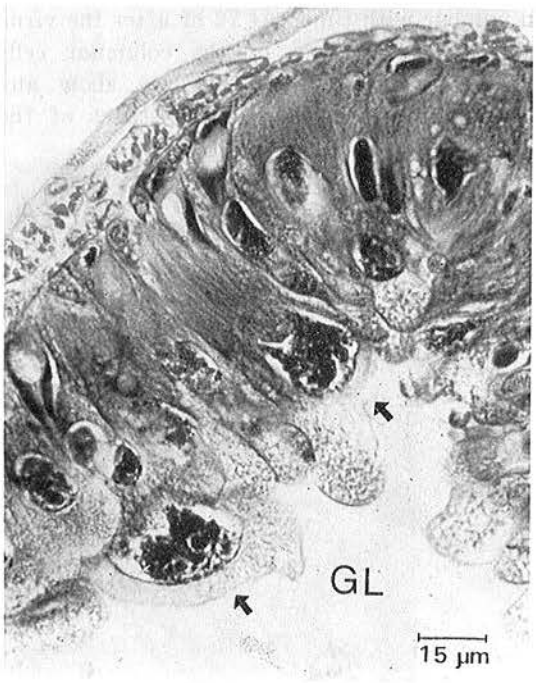

Plate 1. Cross section of midgut epithelium of the silkworm larva infected with DNV. Arrows indicate the infected columnar cell with hypertrophied nucleus discharging into the gut lumen (GL).

ture is quite comparable to that of the infected midgut with IFV, where goblet cells are first degenerated.

A light-radioautographic study[2] with ^{3}H-thymidine revealed that DNA synthesis occurred predominantly in the infected nucleus of columnar cell, indicating the virus seemed to be a DNA virus multiplying in the nucleus. Electron micrograph showed that spherical virus particles multiplied in the nucleus of the columnar cell, while the adjacent goblet cells showed no infection.

Further, the infection and multiplication site of a densonucleosis virus in the silkworm were studied by means of the fluorescent antibody technique.[3] Among various tissues of the infected larvae, only the midgut epithelium showed specific fluorescence. The fluorescence was restricted to the nuclei of the columnar cells, showing the site of virus multiplication. The fluorescence appeared sporadically in a few columnar cells 24 hr after virus administration and became more intense and increased in number with time. At 72 hr after the virus administration, most of the columnar cells near the cardiac valve did not show any fluorescence even in the later stages of the disease.

The multiplication of DNV was greatly reduced when the infected larvae reared at 25–28°C were transferred to an environment at the high temperature of 37°C.[4] Autoradiographic results with ^{3}H-thymidine and ^{3}H-tyrosine revealed that the synthesis of both viral DNA and protein was greatly reduced in the infected larvae maintained at 37°C. Fluorescent antibody studies also confirmed that the synthesis of DNV-antigen in the larva was inhibited at 37°C. These results indicated that high temperature (37°C) may reduce the activity of enzymes concerned with viral DNA and protein synthesis.

Physical and chemical natures of the virus

For purification of *Bombyx* DNV from the infected midgut, a standard procedure with ultracentrifugation of the supernatant of mercerated diseased midgut on 50% sucrose and then 10–40% sucrose gradient was established.[5] Thus purified virus is a spherical particle with a diameter of about 21 nm (Plate 2). Serological studies with the anti-

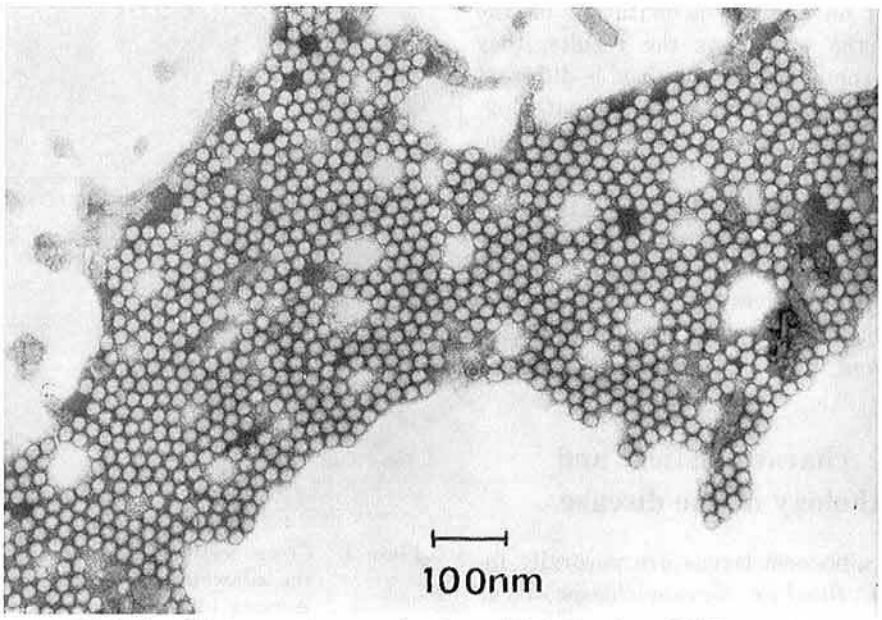

Plate 2. Electron micrograph of purified *Bombyx* DNV particles, negatively stained with 2% PTA.

serum of purified *Bombyx* DNV revealed that the virus is serologically quite different from an IFV of the silkworm, as well as DNVs isolated from *G. mellonella* and *Junonia coenia*.

The extracted nucleic acid from the virus was confirmed to be DNA according to the positive reaction to diphenylamine and the following base ratio; G:A:C:T=22.1:30.0:20.6:27.2[6]. Characterization studies[7,8] of the virus DNA revealed that *Bombyx* DNV had a sedimentation coefficient of 102 s and contained 28 ± 2% of DNA. The DNA in low-salt buffer possessed properties typical of a single-stranded (ss) molecule. Double-stranded (ds) DNA was extracted under conditions of appropriate high salt and elevated temperatures. Electron micrograph of the ds DNA revealed that it was composed of linear molecules with an average length of 1.7 μm and other less well-defined structures. The linear ds molecule had a molecular weight of about 3.4×10^6 determined by electron microscopy and agarose gel electrophoresis. When the ds DNA was alkali-denatured and examined under electron microscope, linear ss molecule with appropriate length of 1.7 μm observed, indicating that the linear ss molecules of unit length.

Four structural proteins were found in purified *Bombyx* DNP which were analyzed by SDS-polyacrylamide gel electrophoresis.[9] The major viral protein (VP1), accounting for 65% of the total virion protein, had a molecular weight of about 50,000, and the other three minor proteins (VP2, VP3, VP4) had molecular weights of about 57,000, 70,000, and 77,000, respectively. The *Bombyx* DNV particle contained about 60 molecules of VP1, and VPI was believed to be capsid protein.

From the physical and chemical natures of the virus mentioned above, *Bombyx* DNV belongs taxonomically to *Densovirus*, a genus of the family Parvoviridae.

Silkworm resistance to DNV infection

Peroral inoculation tests of DNV to various silkworm strains revealed that most of the silkworm strains were nonsusceptible, while some other strains were highly susceptible to the DNV infection.[10] In order to determine the mode of interitance of the nonsusceptibility, the infection tests with the susceptible (S) and nonsusceptible (N) parent strains, their reciprocal F_1 hybrids and the backcrossed hybrids to either of the parents were made. The -log IC_{50} (median infective concentration of virus) values of reciprocal F_1 hybrids and those of their backcrossed hybrid to the S strain were nearly the same as that of the S strain, while the -log IC_{50} value of F_2 hybrid was a little less, and the value of the backcrossed hybrid to the N strain was significantly less than that of the S strain. When DNV of high concentration that resulted in 100% infection in the S strain was administered to the F_2 hybrid and the backcrossed hybrid to the N strain, the susceptible and nonsusceptible larvae were segregated at a 3:1 and a 1:1 ratio, respectively.

These results indicated that the nonsusceptibility to DNV infection was controlled genetically by a recessive gene which was not sex-linked. Therefore, practical rearing of the silkworm strain which contains homozygously the nonsusceptibility recessive gene seems to be one of the best ways for protection against epizootics of densonucleosis in sericultural farms. The biochemical mechanism by which the nonsusceptibility gene caused resistance to DNV in the silkworm is unknown. But it has been speculated that the recessive nonsusceptibility gene may control one of the host cell enzymes that is concerned with DNV replication or with receptor synthesis.

Epizootiology of densonucleosis in the silkworm

Epizootiological investigations were made[11] on the occurrence of densonucleosis at sericultural farms in two separated districts, Saitama and Nagano prefectures, indicating an enzootic of densonucleosis in the both sericultural areas; occurrence of the disease was

only noted at a few farms in Nagano Prefecture. The enzootic was mainly due to the rearing of the silkworm strains of nonsusceptible or highly resistant to the DNV infection. However, DNV was generally detected in the dust from every farm in Nagano Prefecture, even from farms where nonsusceptible silkworm strains were reared and no occurrence of densonucleosis had been seen.

It was found that the DNV detected in the dust was derived from contaminated mulberry leaves with a virus which multiplied chronically in the mulberry pyralid, *Glyphodes pyloalis*, infesting the mulberry plantation. The virus multiplied in the midgut of the mulberry pyralid was not distinguishable in serological natures from *Bombyx* DNV, nor in the pathogenicity to the susceptible silkworm. In Saitama Prefecture, on the other hand, the DNV was not detected in the dust from any farm, nor larva of mulberry pyralid contained DNV in the midgut was recognized.

These results suggest that the epizootic of densonucleosis in sericultural farms is caused by the two major factors; rearing of susceptible silkworm strains in sericultural farms and infestation of mulberry pyralid containing DNV in the mulberry plantation. The results also suggest that the virus isolated in 1968 as *Bombyx* DNV is originally derived from a virus of the mulberry pyralid. However, comparative studies of chemical and physical natures between the both viruses is further required to get information for genetical variation and micro-evolution of insect viruses.

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