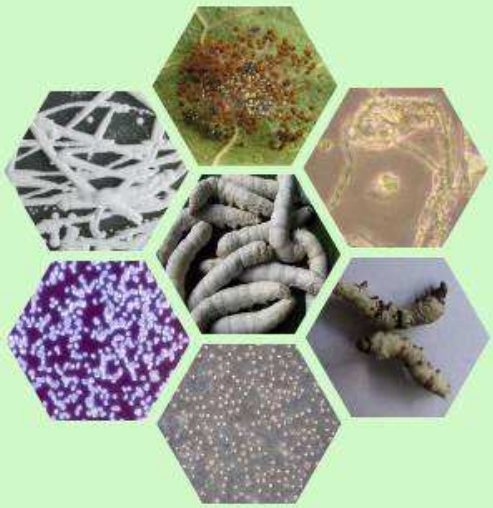


# TITLES AND ABSTRACTS ON SILKWORM DISEASES

*Compiled from*

# THE JOURNAL OF SERICULTURE SCIENCE OF JAPAN

1951-2004 (Vol. 20-73)

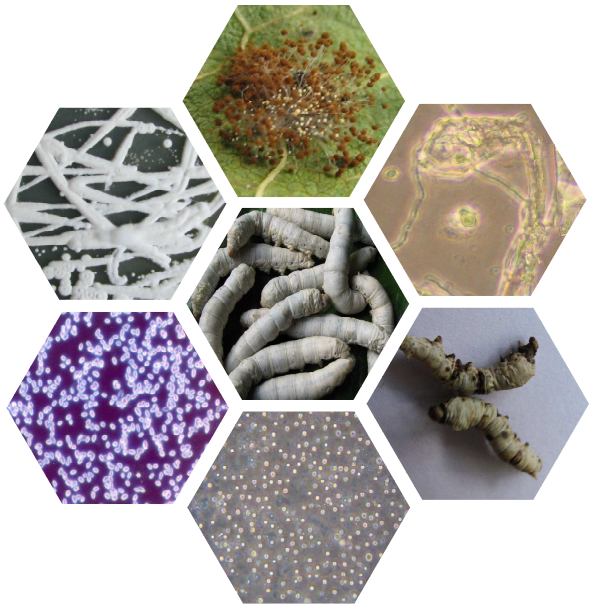
J. JUSTIN KUMAR

# TITLES AND ABSTRACTS ON SILKWORM DISEASES

*Compiled from*

# THE JOURNAL OF SERICULURAL SCIENCE OF JAPAN

## 1951-2004




Compiled & Edited

J. JUSTIN KUMAR

**TITLES AND ABSTRACTS ON SILKWORM DISEASES**

***Compiled from***

**THE JOURNAL OF SERICULURAL SCIENCE OF JAPAN 1951-2004**

**July 2008**

***Compiled & Edited***

**J. Justin Kumar**
Technical Assistant
CSRTI, Central Silk Board
Srirampura, Mysore, Karnataka 570 008.
E-mail: **justinkumarj@gmail.com** Mob: **+91 9449942671**

***Book Lay-out & Design***

**J. Justin Kumar**

# *Preface*

Understanding the silkworm diseases to overcome the problem of crop loss is a basic requirement for better productivity in sericulture. In this direction, a lot of research has been carried out especially in the countries where sericulture is practised seriously. While scanning through the literature, it was observed that, a lot of work has been carried out in Japan which is well documented, mostly in Japanese language. Due to language constraint, it is rather difficult to fully understand the works of Japanese scientists. However, the direction in which they contributed in the field of silkworm pathology is quite interesting. As a curious student, I made an attempt to compile the titles and abstracts of the publications appeared in the Journal of Sericultural Science of Japan with respect to silkworm pathology from 1951 (Vol. 20) to 2004 (Vol. 73). The volumes available at the libraries of Silkworm Seed Technology Laboratory (SSTL), Bangalore and Central Sericultural Research and Training Institute (CSRTI), Mysore were referred to in the preparation of this compilation. The articles in Japanese language with titles in English are indicated as Japanese and full length articles in English language are indicated as English. No indications are given for the articles where the full length paper is in Japanese and titles and abstracts are in English. I earnestly hope that this compilation will be of immense use to the researchers and students working in the field of silkworm pathology.

I am highly indebted to all those who have helped me in this endeavour. The support by the library officials of SSTL, Bangalore and CSRTI, Mysore was tremendous. I thank Mr. Mathew John, who provided me with all the facilities including his computer for initiating this work during my stay at Bangalore.

I am thankful to my wife Letha and daughter Akshaya for their moral support, sparing their precious time and bearing with me.

Mysore
1st July 2008

J. JUSTIN KUMAR

## Dedicated to

*A country peasant & his wife – my Father & Mother*

# Contents

# TITLES AND ABSTRACTS ON SILKWORM DISEASES

## *Compiled from*

## THE JOURNAL OF SERICULURAL SCIENCE OF JAPAN

## 1951-2004

### Vol. 20 1951

**Yoshitake N, Aruga H (1951). Studies on the amino acids in the silkworm. (VII) On the amino acids in the polyhedral body of the grasserie of the silkworm. *J. Sericult. Sci. Jpn*. 20:264-267. [Japanese]**

**Sato T (1951). On the application of Hammerl's of formaldehyde-gas to the disinfection of Muscardines. *J. Sericult. Sci. Jpn*. 20:357-364. [Japanese]**

**Katsumata F (1951). On the Muscardine of the silkworm in Tanaba Province with special reference to the distribution of the species of fungus and the course of infection. *J. Sericult. Sci. Jpn*. 20:365-367. [Japanese]**

**Mitani K, Kanai T (1951). Studies on the disease caused by *Aspergillus oryzae* in the silkworm. (IV) Influence of temperature on the occurrence of disease. (V) The preventive effect of Benzoinc acid to the growth of causal fungus on the paste, the source of the pathogen. *J. Sericult. Sci. Jpn*. 20:368-372. [Japanese]**

**Kiyoshi Aoki, Yasuo Nakazato, Isao Hudimoto (1951). Studies on the relation between Fungi and Insects. (I) Flora of Muscardines on Various Insects. *J. Sericult. Sci. Jpn*. 20:373-382.**

In this paper muscardines on domesticated silkworms and other wild insects, which were collected at 13 prefectures during the period from 1936 to 1950 were described. 40047 strains of muscardines were found on silkworms and other wild insects of 24 kinds, namely 33504 strains on silkworms and 6543 on wild insects. The muscardines isolated from domesticated silkworms were divided into 7 species which are hitherto known, 1 species of new pathogen and 1 genus which is not yet identified the species. White muscardine was recognized frequently and in abundance and the frequency in occurrence of green and brown muscardines followed that of white muscardine, all the other muscardines being rare. Out of 33504 strains, 28938 were *Beauveria bassiana* (Bals.) Vuill. (White muscardine), the other 1957, 1941, 315, 188, 152, 6, 4 and 3 strains being respectively *Nomuraea prasina* Maubl. (Green muscardine), *Aspergillus flavus* Link., (and *A. oryzae* Cohn, Brown muscardine), *Isaria farinosa* (Dicks.) Fr. (Yellow muscardine), *Oospora destructor* (Metch.) Delac. (Black muscardine), *Fusarium* spp., *Sterigmatocystis japonica* Aoki, *Harziella entomophilla* Ishiwata et Miyake and new muscardine.

The muscardines on wild insects of 24 species were divided into 9 species which are already known and 4 genera which are not identified the species. Out of 6543 strains, 3417 were yellow muscardine, the other 1364, 323, 319, 263, 155, 122, 121, 118, 39, 24, 11 and 2 being respectively *Isaria fumoso-rosea* Wize (red muscardine), *Sterigmatocystis japonica* Aoki,

*Fusarium* spp., *Empusa* (*Muscae* Cohn and *Aulicae* Reich), *Aspergillus* (*flavus* and *oryzae*), *Isaria sinclairii* (Berk.) Lloyd., black muscardine, *Harziella* sp. and *Massospora* sp.

Out of 12 species and 4 genera of muscardines on various insects, 5 species ad 1 genus were mutual to silkworm and wild insects. White, red and yellow muscardines were very abundant in occurrence on domesticated silkworms (*Bombyx mori* L.), Kyoso-fly-pupae (*sturmia sericariae* C.) and pyralid moth (*Margaronia pyroalis* W.) respectively. The facts that white and red muscardines which were found abundantly on domesticated silkworm and Kyoso-pupa respectively were not found on wild insects and silkworm and that yellow muscardine, which was prevalent on pyrolid moth, was rare on silkworm and Kyoso-fly-pupa are remarkable. Green muscardine appears to be mutual to silkworms and wild insects. But white muscardine is specific to silkworm. Although white muscardine is found almost invariably regardless of the silkworm larva stage, green muscardine is found chiefly in the third moult and the brown one in the early stage and matured larvae or pupae.

**Aoki K, Nakazato Y, Hudimoto I (1951). Studies on the relation between Fungi and Insects. (II) On the pathogenicity of Muscardines and their growth in the insect blood. *J. Sericult. Sci. Jpn*. 20:430-438.**

The pathogenic activity of white, red and yellow muscardine (*Beauveria bassiana* Vuill., *Isaria fumorosea* Wize and *Isaria farinose* FR respectively) is most vigorous to the host insects on which they are most abundantly found, namely to domesticated silkworm (*Bombyx mori* L.), Kyoso-fly-pupa (*Sturmia sericariae* C.) and pyralid moth (*Margaronia pyroalis* W.) respectively. White muscardine is more pathogenic to domesticated silkworm than to the wild silkworm (*Theophila mandarina* Moore). The pathogenicity of this fungus is also influenced by the races of silkworm.

Although white muscardine is invariably pathogenic to domesticated silkworm regardless of the larval stage, the pathogenicity of green muscardine is conspicuously affected by the stage, being vigorous to the silkworm in earlier stage and faint to the one in late stage. The incubation period of green muscardine is longer than the one of white muscardine. The reason why green muscardine occurs simultaneously in the third moult was discussed.

The pathogenicity of white, red and yellow muscardines to silkworm, Kyoso-fly-pupa and pyralid moth respectively and the one of green muscardine to the various stages of domesticated silkworm were found to be directly proportional to the germinating rate of spores, the growth rate of germ tube and the speed of cylindrical spore formation of the causal fungi in the insect's blood.

## Vol. 21 1952

**Kuwano T (1952). Prevention of Muscardine by application of ceresan. (III) Comparison of lime and talc as a diluting agent of ceresin. *J. Sericult. Sci. Jpn*. 21:101-105. [Japanese]**

**Aizawa K (1952). Preliminary note on the successive passage through *Philosamia ricini* Boisd of jaundice virus of *Bombyx mori*. *J. Sericult. Sci. Jpn*. 21:170-172.**

The successive passages (six generations) of the silkworm jaundice virus in the pupae of *Philosamia ricini* Boisd were performed by the method of Kawakita. Polyhedral bodies of the silkworm jaundice type could be detected in the infected pupae of *P. ricini* Boisd and their blood was infectious to the silkworm.

**Muroga M (1952). Effects of urea on silkworm larva, *Bombyx mori* L. (I) *J. Sericult. Sci. Jpn*. 21:280-282. [Japanese]**

**Sano T (1952). Studies on the silkworm disease caused by *Mermithid nema* (I) *J. Sericult. Sci. Jpn*. 21:298-301. [Japanese]**

## Vol. 22 1953

Nil

## Vol. 23 1954

**Aoki K, Nakazato Y, Hudimoto I and Ishii H (1953). Control of green muscardine caused by *Spicaria prasina* (Maubl.) Aoki *J. Sericult. Sci. Jpn*. 23:108-113. [Japanese]**

**Inagami K (1954). Studies on the proteins of the body fluid in the silkworm (III) On the electrophoretic components of the body fluid proteins. *J. Sericult. Sci. Jpn*. 23:304-307.**

The body fluid of the silkworm (protein solution) has been protected from the melanose by adding CN. This analysis has been carried out by using 0.05m Na2CO3, 0.05m NaHCO3 solution. The body fluid of the silkworm consists of albumin, α globulin and β globulin and does not contain fibrinogen and γ globulin. In the male, albumin is twice as much as α, β globulin but α globulin is found the greatest quantity in the female. By virus infection α, β globulin increases.

## Vol. 24 1955

**Kuwano T (1955). Control of muscardine by means of PM paper (I) Effectiveness to the *Aspergillus. J. Sericult. Sci. Jpn*. 24:145-149. [Japanese]**

**Aoki K, Nakasato Y and Hujimoto I (1955). Control of muscardine by the method of fungicide-paper. *J. Sericult. Sci. Jpn*. 23:150-155.**

Fungicide paper prepared by the dipping of filter paper into the ceresin solution the percentage of ceresin being 0.2~2.0% was tested for the control of white muscardine after the paper has been dried. When the fungicide papers were applied under and over the rearing seat, they were very effective for the prevention of this disease in both the earlier later larval stage by the concentration of from 0.5 to 2.0%. But the effectiveness is not sufficient by the method of covering only the rearing seat. The preventive effect of our fungicide paper seems to be due rather to the repression of germination than the sterilization of the conidia.

## Vol. 25 1956

Nil

## Vol. 26 1957

**Aruga H, Watanabe H, Fukuhara T and Iwashita Y (1957). Mechanism of the virus resistance in the silkworm, *Bombyx mori* (I) On the formation of the polyhedral body in the nucleus of the silkgland cell. *J. Sericult. Sci. Jpn*. 26:105-112.**

**Aruga H (1957). Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori*. (II) On the relation between the nuclear polyhedrosis and the cytoplasmic polyhedrosis. *J. Sericult. Sci. Jpn*. 26:279-283.**

Studies on the nuclear polyhedrosis and the cytoplasmic polyhedrosis (mid-gut polyhedrosis) in the silkworm have been carried out by using several races and their hybrids. It may be plausibly thought that the virus of the cytoplasmic polyhedrosis is different from that of the nuclear type, confirmation being made by feeding experiments with polyhedra plastered mulberry leaves, the position difference in their formation, and the stainability of the polyhedral bodies. In numerous larvae cytoplasmic polyhedrosis could be induced by a low temperature (5°C, 24 h) treatment of the larvae just after ecdysis, particular in the 5$^{th}$ larval stage as in the case of the nuclear polyhedrosis. The midgut polyhedral virus may be imagined to be due to a mutation of the nuclear type, whence the former has acquired the characteristic of multiplying more readily in the cytoplasm of the cylinder cells of the midgut than the midgut polyhedral virus reported by Ishimori (1934).

**Iwashita Y and Aruga H (1957). Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori*. (III) Histological studies on the polyhedrosis in the silkworm. *J. Sericult. Sci. Jpn*. 26:323-328.**

Histological and histochemical studies on the polyhedral diseases, particularly on the relation between the process of the formation of polyhedra and nucleic acid have been carried out with various staining techniques. The results obtained are as follows:

Polyhedral bodies are formed in the cell nuclei of oenocyte, muscle, basement membrane and epithelial cells of the midgut of nuclear type polyhedral diseased larva. The process of the polyhedral formation in the cytoplasm of cylindrical cells of the midgut was investigated. In general the cytoplasmic type polyhedra are formed in the cytoplasm of cylindrical cells, but in few cases are formed in the goblet cells, too. Regenerative cells of the midgut located in *nidi* at the base of the digestive cells are not susceptible to virus infection, but in few of them polyhedra may form when they develop accompanying the degeneration and destruction of the epithelial cells. Cross infection of both the nuclear and the cytoplasmic types was observed in the midgut epithelium of first instar larva. Nuclear type polyhedra showed a positive feulgen reaction, indicating the presence of de-oxyribose nucleic acid (DNA), but cytoplasmic ones showed a negative reaction. The stainability difference between the nuclear and cytoplasmic polyhedra was investigated by applying a new method of staining with orange G-aniline blue.

**Ishimori N (1957). Review on the studies of polyhedrosis in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 26:412-418 [Japanese]**

## Vol. 27 1958

**Aruga H (1958). Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori* (IV) On the relation between the polyhedral diseases and environmental factors. *J. Sericult. Sci. Jpn*. 27:5-9.**

A comparative study on the percentage of appearance of cytoplasmic and nuclear polyhedrosis in the silkworm reared at University of Tokyo and Shinshu University, (Ueda, Nagano Prefecture) based upon the probable difference in the quality of mulberry leaves grown at the two respective places, using several resistant and susceptible strains and their hybrids, was made. The results obtained are summarized as follows:

In the experiments conducted on the spring rearing, in which the low temperature treatment (5°C, 24 h) was given to the 5$^{th}$ instar soon after ecdysis, cytoplasmic (midgut) polyhedral diseased larvae appeared less at Ueda than at Tokyo. In the case of summer rearing in which low temperature treatment was not applied, a result similar to the above was obtained, ie, the number of larvae affected by midgut polyhedral disease were less in Ueda than at Tokyo. The percentage

of midgut polyhedral diseased larvae induced by the low temperature treatment in the summer rearing was higher at Tokyo than at Ueda. The larvae reared first at Ueda for the 1st and 2nd instar and then at Tokyo from 3rd to 5th instar, showed an intermediate value of midgut polyhedrosis between those reared at Ueda and at Tokyo, respectively from the 1st upto 5th instar. From these results, it may be plausibly thought that some environmental factors, in this case the quality of mulberry leaves, markedly influence the appearance and induction of the cytoplasmic polyhedrosis in the silkworm.

**Aruga H (1958). Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori* (V) On the incidence of polyhedral viruses by nitrogen mustard. *J. Sericult. Sci. Jpn*. 27:10-13.**

Studies on the induction of nuclear and cytoplasmic (midgut) polyhedral viruses utilizing nitrogen mustard, X-rays, ultraviolet rays have been carried out in the silkworm with a few susceptible and resistant races, hybrids and *od* oily mutant. As numerous diseased larvae in the 5th instar appeared typically displaying the formation of polyhedral bodies in the cytoplasm of cylindrical cells in midgut by the nitrogen mustard treatment (feeding and injection) in the 3rd, 4th and 5th instars, so it may be concluded that the cytoplasmic polyhedral virus can be induced by the nitrogen mustard treatment. The nuclear polyhedral bodies observed in a hybrid treated by nitrogen mustard showed tetragonal configuration different from normal polyhedra, which are hexagonal. The phenomenon of the induction of both the nuclear and cytoplasmic polyhedral viruses was not recognized in the experiments of X-rays and ultraviolet ray treatments.

**Aruga H (1958). Mechanism of resistance to virus diseases in the silkworm, *Bombyx mori* (VI) On the relation between the rearing season and the cytoplasmic polyhedrosis. *J. Sericult. Sci. Jpn*. 27:14-17.**

Studies to investigate the relationship between the occurrence of the cytoplasmic polyhedrosis (midgut polyhedral disease) and the rearing seasons (spring, summer and autumn) in which the quality of mulberry was thought to be different, have been made in these three years (1955-1957). These experiments have been carried out both with no treatment and treatment with low temperature (5°C, 24 h) of the 5th instar larvae soon after ecdysis using numerous races, hybrids and biochemical mutants.

In the spring rearing, much less mid-gut polyhedral diseased larvae occurred than in the summer and late autumn seasons. The midgut polyhedrosis showed less occurrence in summer rearing than in the late autumn. The same tendency was observable in the induction experiments of the midgut polyhedral virus by the low temperature treatment. It may be plausibly thought that the main factor controlling the occurrence of the midgut polyhedrosis is the quality of mulberry leaf. But several other environmental factors like temperature, humidity, etc, should not be ignored. The quality of mulberry leaf given to the larvae before the low temperature treatment has the influence upon the induction of the midgut polyhedral virus.

**Shigematsu H and Takashita H (1958). Changes in quantity of nucleic acid and protein in the fat body of the silkworm in a course of contracting jaundice. *J. Sericult. Sci. Jpn*. 27:66-70.**

Changes in quantity of nucleic acid and proteins in the fat body of the silkworm were investigated from the day of intracutaneous inoculation of jaundice virus to the death, in order to find a functional alternation of the tissue with the multiplication of viruses and of polyhedral bodies. Efficiency of extraction of these substances with cold 0.85% KCl solution was proposed. Total nucleic acid was extracted with 2M NaCl (pH 7.4) in boiling water bath for one hour. The acid extracted was confirmed with absorption spectrum.

The content of nucleic acid and protein of the fat body fluctuated in the same manner in the following three fractions; whole tissue, KCl supernatant and its precipitate. In the early time of the infection which is to be the period of virus multiplication, no difference was detected in quantity of these substances between the inoculated and the control. The difference was observed about four days after the inoculation when the body fluid became turbid with polyhedral bodies. The mode of change in content of these substances in body fluid was the same as that in the fat body.

**Sasamoto K and Muramastus N (1958). On the relation between muscardine of wild insects and of silkworm. *J. Sericult. Sci. Jpn*. 27:76-80.**

The muscardines on both silkworms and wild insects were almost *Isaria farinosa* (DICKS.) FR. and *Isaria* sp. in Yamanashi Prefecture in 1953~1954. It was proved that the fungi on wild insects showed a severe pathogenicity to the silkworm. So the writers tried to seek the course of infection in order to get preventive measures against this muscardine and got the following results.

The longevity of spores of *Isaria farinosa* is comparatively short and has little strength to kill silkworms in next spring. But the mycelia hibernate in wild insects, such as *Lyprops sinensis* MARSEUL and *Dendrolimus spectabilis* BUTLER which are apt to be affected by the same fungus, without losing the activity for 9 months or more. In the next spring they form the spores in the moderate temperature and moisture. These spores kill silkworms in the spring and give bad influence to future sericulture yieldings. The yellow muscardines of the late autumn which were cast in the shade or remained in the rearing room reform spores in the next spring, so we should clear away them perfectly in the autumn. The spores of *Isaria farinosa* stick to mulberry leaves and enter into the rearing room. Then they cause the disease. So the silkworms and rearing bed should be sterilized frequency.

**Ishikawa S (1958). On the respiratory enzymes in the midgut of the cytoplasmic polyhedrosis silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 27:99-103.**

Respiratory enzyme activities in the homogenate and the mitochondria of the midgut of *Bombyx* larva were measured both with cytoplasmic polyhedrosis diseased and with healthy worms. The results obtained were as follows:

Inhibition rate of endogenous respiration of the diseased homogenate by 0.01 M EDTA is less than that of the healthy one. Both in the healthy and the diseased worms, the EDTA inhibition was recovered by adding such metals as $Mg^{++}$, $Mn^{++}$, $Co^{++}$ and $Fe^{+++}$ with the exception of $Cu^{++}$. The rates of the recovery differ between the healthy and the diseased ones, in the former the recovery does not reach the level of normal endogenous respiration but in the latter it is remarkable. Both activities of cytochrome oxidase and succinic dehydrogenase in the mitochondria did not show any difference between the healthy and the diseased worms. Oxidation of succinate, malate and glutamate by both the homogenate and mitochondria of the diseased worm were lower than those in the healthy ones. Activation by adding DPN on the glutamate oxidation in the mitochondria of the diseased was less than that in the healthy. The same relation was found in the case of activation by adding ATP on the succinate oxidation.

**Iwashita Y (1958). The process of the crystalline substances formation in the nucleus of cylindrical cells in the midgut of the cytoplasmic polyhedrosis disease of the silkworm. *J. Sericult. Sci. Jpn*. 27:107-110.**

Some crystalline substances are formed in the nuclei of some cylindrical cells in the midgut of the cytoplasmic polyhedral diseased silkworms. The histological and cytological

investigations have been carried out concerning the formation and stainability of these crystalline substances. The results obtained are as follows:

The crystalline substances are usually square in form, but some are round or polygonal, and are 6~10 micron in diameter. The crystalline substances are acidophil and particularly can be stained well with orange G, eosin, phloxin or bromophenol blue after they are hydrolyzed in 1 N HCl at 60°C for several minutes. However, they can not be stained with pironin, haematoxylin or through Feulgen reaction. It is most probable that the formation of these crystalline substances may have some relation with the nucleolus, and that the nuclei of the infected cylindrical cells continue to produce the nuclear substances which lead to the formation of these crystalline substances even in the process of the polyhedral formation in the cytoplasm, and that some of these nuclear substances are crystallized in the nucleus, keeping a close relation with the nucleolus.

**Aoki K, Nakazato T, Ishiie T and Suzuki H (1958). Destruction of *Aspergillus oryzae* invased in wooden parts of rearing tools by some systemic fungicides and the duration period of their preventive effect. *J. Sericult. Sci. Jpn*. 27:337-341.**

The present paper dealt with the effect of some systemic fungicides on the destruction of *Aspergillus oryzae* invased in wooden parts of rearing tools as well and as the duration period of their preventive effect. The systemic fungicides (PCP 400x, PMF 4000x) were sure to control the causal fungus invased in the wooden parts by the dipping for 15 minutes. Some usual disinfectants (chloride of lime 100x, formalin 7x) lost their preventive effects to the fungus invasion 24 h after the disinfection of the wooden parts. The duration of the residual effect to the invasion is 100 days or more by PCP 800x or about 80 days by PMF 100x both on pine and Japanese cedar boards, and 100 days or more by PCP 200x or about 25 days by PMF 1000x on bamboo stalks. In the case of use of PCP 400x or PMF 2000x, their effective dipping times for the prevention to the invasion by the causal fungus are 5 minutes on pine and Japanese cedar boards, and 15 minutes on bamboo stalks.

## Vol. 28 1959

**Kitazawa T and Takami T (1959). Inoculation of silkworm embryos with the intestinal cytoplasmic polyhedral virus. *J. Sericult. Sci. Jpn*. 28:59-64. [Japanese]**

**Takami T and Kitazawa T (1959). Inoculation of fasting newly hatched silkworms with the silkworm jaundice virus (Preliminary report). *J. Sericult. Sci. Jpn*. 28:65-66. [Japanese]**

**Miyoshi T (1959). Physiological studies on the flacherie of the silkworm, *Bombyx mori* (I) Changes in the content of free amino acids and the refractive index of body fluid according to different stages, sexes, races and health conditions of lmother population in the silkworm. *J. Sericult. Sci. Jpn*. 28:88-93.**

To clarify the physiological aspects of flacherie in the silkworm, *Bombyx mori*, a series of experiments was designed. The content of free amino acids in the body fluid from the 3$^{rd}$ instar larvae to pupal stage was estimated in the spring, early autumn and autumn seasons, and the author tried the comparison of the content among sexes, rearing seasons, races, developmental stages and health conditions. The results obtained are as follows:

The free amino acids contents in body fluid was maximum at the periods just before moulting in the 4$^{th}$ instar and before spinning in the 5$^{th}$ instar. The maximum value of amino-N in the 3$^{rd}$ instar was nearly the same as that in the 4$^{th}$ instar, about 3.0 mg per ml of body fluid, but in the 5$^{th}$ instar it showed a tendency to decrease a little (2.5 mg per ml of body fluid). On the whole it was found that the amino-N consistency in the 4$^{th}$ instar is nearly the same as that in the

3rd instar, but is higher than that in the 5th instar. It was found that the content of amino-N scarcely differed between male and female up to the middle stage of 5th instar, but afterwards female exceeded a little, but steadily over male, and the difference extended after spinning. From the results obtained it may be plausibly thought that the content of free amino acids in body fluid has no correlation with rearing seasons, races and health of mother population. The refractive index of body fluid, different from the case of amino-N content, increased from the 3rd instar to the 5th instar and in the same instar, like amino-N content, it reached to maximum at the end of the instar decreasing slowly during molting. The refractive index too seems to have no correlation with the rearing seasons, races and health of mother population.

**Miyoshi T (1959). Physiological studies on the flacherie of the silkworm, *Bombyx mori* (II) The content of free amino acids and the refractive index of body fluid in flacherie and starved silkworms. *J. Sericult. Sci. Jpn*. 28:94-97.**

In the first paper of this series attention has been drawn to the variation of the content of free amino acids in the body fluid of healthy larvae of the silkworm. The purpose of the present investigation is to compare the content of free amino acids in blood among flacherie, starved and healthy silkworm larvae at the same stage and sex. The results obtained are as follows:

Scarcely or no difference is observable in the content of free amino-N in the body fluid between healthy and starved (for 96 h) larvae, whereas the content is considerably less in flacherie individuals than in starved ones. Refractive index of the body fluid of starved and flacherie larvae is nearly the same. From the above mentioned results it may be concluded that the physiological abnormality occurring in flacherie individuals is not simply attributed to starvation but can rather be caused by other factors also.

**Hukuhara T and Aruga H (1959). Induction of polyhedroses by temperature treatment in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 28:235-241.**

When fifth instar larvae of the silkworm before first feeding after ecdysis are subjected to low temperature (0-3°C) for 16 or 24 h, both nuclear and cytoplasmic (mid-gut) polyhedrosis break out with much higher frequency than that in the control. Intermittent cold treatment is more effective than continuous one in induction of polyhedrosis. Silkworm stage plays an important part in the efficiency of induction by cold-treatment. Aptitude is low during moult, whereas after ecdysis it rapidly increases and then reaches a very high level, which is maintained for certain period. Larvae fed with mulberry leaves after ecdysis show remarkably low aptitude. The results obtained by partial heat treatment of larval body reveal that high temperature can induce the polyhedrosis and special organs or tissues situated in the anterior part of larval body play a part in the induction of polyhedrosis. The process of induction could be visualized as follows. Temperature treatment disturbs the physiological condition of larvae, culminating in an abnormal condition, which in turn causes the transformation of the viruses from a non-infective state to an infective one.

**Aruga H and Watanabe H (1959). Difference of induction rate of polyhedroses by low temperature treatment between inbred lines and their hybrids in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 28:302-307.**

Among various inbred strains of the silkworm, Daizo and Shungetsu are resistant to both nuclear and cytoplasmic (mid-gut) polyhedrosis, while Ryo and Shuka re susceptible to both polyhedrosis, especially cytoplasmic one. Percentages of polyhedrosis in the fifth instar larvae of these four inbred strains and their F1 hybrids, as well as both F2 and backcrossed hybrids, were measured. Each percentage of polyhedrosis was converted into MATHER'S potence for the sake of ready comparison of the resistance between hybrids and their parental lines.

This results obtained from the test, under the ordinary rearing conditions, indicated that frequency of polyhedroses in F1 hybrids is much less than their parental lines and F1 hybrids show heterosis for the resistance to the two kinds of polyhedroses. The results also suggested that the amount of heterosis for the resistance to cytoplasmic polyhedrosis is liable to be larger then for the resistance to nuclear polyhedrosis. The heterosis for the resistance was generally speacking, also observable in F2 and backcrossed generations, though the amount was less than in F1 hybrids.

**Ishikawa Y and Asayama T (1959). On the translocation of the cytoplasmic polyhedral bodies in the midgut during metamorphosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 28:308-312. [Japanese]**

**Aruga H and Arai N (1959). Studies on the induction of polyhedroses by the low temperature treatment in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 28:362-368.**

Studies on the induction of nuclear and cytoplasmic polyhedroses by the cold treatment were carried out using the silkworm larvae of several developmental stages in the spring and summer seasons. The results obtained are as follows;

No induction phenomena were observed in the larvae of the 1st, 2nd and 3rd moulting stages and the larvae soon after ecdysis of the 2nd and 3rd instar by the cold treatment (5°C, 24 h) but induction was recognized in the larvae of the 4th moulting and larvae soon after ecdysis of the 4th and 5th instars, showing the highest induction rate in the stage of the 5th instar soon after ecdysis. Cytoplasmic polyhedrosis was considerably induced by cold treatment for 6 h carried in the 5th instar larvae soon after ecdysis. The polyhedral disease was induced in the considerably high rate in several stages of the 4th and 5th instars except for the cold treatment of the later stages of the 6th day of 5th instar. It is interesting that the induction of the cytoplasmic polyhedrosis was recognized not only in the stage of no feeding but also in several stages of feeding of mulberry leaves. Nuclear polyhedrosis was induced by the cold treatment in the stages soon after ecdysis and of the 1st day and from the 5th to 6th day after ecdysis of the 5th instar. In the 5th instar, the induction experiments by cold treatment were carried out to clarify the relationship between the length of starvation after ecdysis and the rate of induction. The rate of induction of both nuclear and cytoplasmic polyhedroses of the larvae treated by the low temperature for 0-6 h after ecdysis was lower than that of the larvae treated for 6-48 h after ecdysis. From the above mentioned results, it is plausibly thought that the rate of induction by the cold treatment may be influenced by the change of physiological function accompanying with the lapse of time after the 4th moult, and this physiological change might be controlled by some hormonal substances.

**Aruga H, Kanai E and Israngkul A (1959). On the relationship between physiological conditions of larvae and the induction of polyhedroses in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 28:369-374.**

It may be considered that genetical, physiological and environmental factors are closely related to the appearance of both nuclear and cytoplasmic polyhedroses in the silkworm. Cold treatment for 24 h to the 5th instar larvae before first feeding after ecdysis induces those polyhedroses with high rate. It has been ascertained that the rate of the appearance of both polyhedroses in natural condition was changed by difference of some nutritional factors having relation with the quality of mulberry leaves and the same phenomenon was observed in the experiments of the artificial induction carried out by changing nutritional factors before and after cold treatment.

As to the relationship between the nutritional conditions of larvae before refrigeration and the induction rate of polyhedroses, the results showed that when nutritional factors of larvae before refrigeration were considered to be relatively good the appearance of nuclear

polyhedroses was high, and that of cytoplasmic one was low, but if the nutritional factors were relatively bad, the tendency of the induction of polyhedroses showed the reversed relation. On the relation between the nutritional conditions of larvae after refrigeration and the induction of nuclear polyhedrosis good conditions of the factors showed that the higher induction rate than the bad ones. From the above mentioned results it might be plausibly thought that the physiological conditions having relation with the nutrition of the silkworm larva play an important role in the spontaneous appearance, and the induction by the cold treatment, of polyhedroses.

## Vol. 29 1960

**Aruga H and Hukuhara T (1960). Induction of nuclear and cytoplasmic polyhedrosis by feeding of some chemicals in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 29:44-49.**

Eighteen chemicals were tested for inducing effect of nuclear and cytoplasmic polyhedrosis by feeding them to fifth instar larvae of silkworm. Among them 6 were found to be effective in the induction of cytoplasmic polyhedrosis. They are sodium cyanide, sodium fluoride, arsenic acid, monojodoacetic acid, sodium azide, ethylene diamine tetraacetic acid (EDTA) and its disodium salt. The last two chemicals induced cytoplasmic polyhedrosis with high frequency when they were fed to fifth instar larvae but only with low frequency when fed to fourth instar larvae. In the induction by these two chemicals, the larvae reared in Tokyo developed only cytoplasmic polyhedrosis, however, those reared in Ueda developed both nuclear and cytoplasmic polyhedrosis. When cold treatment was carried out on fourth instar larvae that had been fed with disodium salt of EDTA, sodium cyanide or mercuric chloride during third instar, they developed cytoplasmic polyhedrosis with higher frequency than the control larvae.

**Yokokawa S and Okabe F (1960). On the cytoplasmic polyhedrosis in larvae caused by inadequate treatment of eggs in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 29:125-128. [Japanese]**

**Yokokawa S and Mochida M (1960). On the occurrence of the cytoplasmic polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 29:129-132. [Japanese]**

**Yokokawa S and Yamaguchi Y (1960). On the induction of the cytoplasmic polyhedrosis in the silkworm larvae by feeding on mulberry leaves with some agricultural chemicals. *J. Sericult. Sci. Jpn*. 29:133-136. [Japanese]**

**Ishikawa Y (1960). Studies on the relation between the polyhedrosis of wild insects and the silkworm, *Bombyx mori* L. (I) On the nuclear polyhedrosis of *Dictyoploca japonica* B. *J. Sericult. Sci. Jpn*. 29:137-140. [Japanese]**

**Harikuza M, Kobayashi M and Yamashita Y (1960). Histopathological and histochemical observations on mid-intestine in flacherie diseased silkworm (*Bombyx mori* L). *J. Sericult. Sci. Jpn*. 29:153-161.**

The authors performed histopathological as well as histochemical observations on three portions, anterior, middle and posterior of the mid-intestine in healthy and flacherie diseased silkworm larvae. In anterior portion of the mid intestine, nuclei in the cylindrical cells in the healthy larvae are situated near of striated border while those in the diseased larvae are positioned near of the basement membrane. And a secretory activity on cylindrical cells of the healthy larva is higher than that of the diseased larva. Each goblet in the healthy larva is vacant, even though that in the diseased larva is full of many secretory materials. In middle portion of the mid-intestine, nuclei in the cylindrical cells of the healthy larva are situated in cytoplasm near of striated border while those of the diseased larva are situated in about middle part of cytoplasm.

The secretory activity of cylindrical cells in both portions, middle and posterior, of the healthy larva is higher than that in the diseased larva.

Each positive reaction of glucose, glycogen, hyaluronic acid, carbohydrates, lipids, DNA and RNA is shown in the mid intestine of both healthy and diseased larvae, while negative reaction of chondrotin sulphuric acid is shown in both. Among those the reactions of glucose, DNA and RNA in the healthy larva are much stronger than those in the diseased larva.

**Aizawa K and Choraku I (1960). Some characteristics of the cytoplasmic polyhedrosis in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 29:363-368.**

Silkworm larvae heavily infected with CPV were diagnosed under in optical microscope and the amount of NPV in the blood or in the midgut was measured. In most cases the NPV was not found, but in a very few cases, a small amount of the virus was found both in blood and in midgut. The latter cases are clearly double infection, which is in the early development of nuclear polyhedrosis. After this examination, cytoplasmic polyhedra were collected from midgut and were fed on the newly hatched larvae, but only cytoplasmic polyhedrosis occurred. The same result was obtained in the feeding experiments of cytoplasmic polyhedra obtained from the digestive fluid of larvae infected with cytoplasmic polyhedrosis. The nuclear polyhedrosis virus was not found in the digestive fluid. Such cytoplasmic polyhedra were dissolved in dilute alkaline solution and were injected subcutaneously into silkworm pupae, but no occurrence of nuclear polyhedrosis was observed.

From these results, it is considered that there is no relation between cytoplasmic and nuclear polyhedrosis viruses from the point of view of infectivity. Spherical viruses like particles, 60-70 mμ in diameter, were seen in the cytoplasmic polyhedra after treatment with dilute $Na_2CO_3$ solution and were purified by differential centrifugation. Cytoplasmic polyhedra were treated with 0.1% $HgCl_2$, 3% phenol, 2% formalin or heat (100°C and 60°c) and it was shown by mean of feeding experiment that the virus was inactivated nearly completely by 2% formalin for 5 h or at 100°c for 5 min.

**Aoki J (1960). Studies on the infection mechanism of *Aspergillus* diseases in silkworm larvae, *Bombyx mori*. (I) Growth and ill condition of infected larvae. *J. Sericult. Sci. Jpn*. 29:425-430.**

By using larvae artificially infected with *Aspergillus flavus-oryzae* group soon after the first molting, observations were made on the duration of the second and the third instars, the second and third sleeping periods and on the mortalities during the feeding as well as the sleeping period. As the results of this exp it was concluded that the duration of the second instar of infected larvae was only slightly retarded when compared with normal ones and on the contrary, it was generally prolonged in the third instar. As for the sleeping period, no difference was observed comparing with the check in the second sleeping period, while it was prolonged in the third one.

The sleeping ratio of infected larvae did not decrease when compared with the check save for the cases with high concentration of fungus suspension in the second instar, whereas it decreased to one half of the check in the third instar. The mortality was similar during the second and the third sleeping periods in the infected larvae, which was larger, in both cases, than the check. Inoculated larvae usually could never sleep or died during the sleep and, even in the rare cases of surviving the sleep, they perished with the shrinkage of the body length in serious condition, while in light condition they were usually kept alive during the next feeding period followed by the death during the sleeping period. As was mentioned we could with difficulties find any incubation period in this disease. The relative live weight of inoculated larvae to normal ones began to diminish three days after the infection, remained constant during the second sleep

and again diminished rapidly from the beginning of the third instar. The second sleep again diminished rapidly from the beginning of the third instar. The diminishing degree in inoculated larvae was more remarkable than the larvae of Muscardine. Also the amount of diet and feces began to diminish from three days after the infection and tends to become more distinguished in the third instar.

**Tanaka S (1960). Studies on the polyhedrosis of *Antheraea yamamai* and *Antheraea pernyi*. (I) On the relationship between the polyhedrosis of *Antheraea pernyi* and that of *Bombyx mori*. *J. Sericult. Sci. Jpn*. 29:431-435. [Japanese]**

**Ishikawa Y and Asayama T (1960). Studies on the relation between the polyhedroses wild insects and the silkworm, *Bombyx mori* L. (II) On the nuclear polyhedrosis of *Philosamia cynthia pryeri* B. *J. Sericult. Sci. Jpn*. 29:506-508. [Japanese]**

**Harizuka M, Nshimura H, Saito C and Oba H (1960). Studies on rearing conditions of *Bombyx* silkworm with special reference to the occurrence of flacherie diseases (I) On rearing conditions during the first and second larval instars. *J. Sericult. Sci. Jpn*. 29:509-514. [Japanese]**

**Harizuka M, Nshimura H, Saito C and Oba H (1960). Studies on rearing conditions of *Bombyx* silkworm with special reference to the occurrence of flacherie diseases (II) On rearing conditions during the third and fourth larval instars. *J. Sericult. Sci. Jpn*. 29:515-518. [Japanese]**

**Harizuka M, Nshimura H, Saito C, Oba H and Takasu T (1960). Studies on rearing conditions of *Bombyx* silkworm with special reference to the occurrence of flacherie diseases (III) On some combinations between rearing conditions of younger larval stages and those of older larval stages. *J. Sericult. Sci. Jpn*. 29:519-524. [Japanese]**

## Vol. 30 1961

**Aruga H, Hukuhara T, Yoshitake N and Israngkul A (1961). Interference between two inoculated viruses of the mid-gut polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:23-30.**

Interference was observed between two cytoplasmic polyhedrosis viruses forming tetragonal and hexagonal polyhedra in the silkworm, *Bombyx mori*, when the two viruses were fed to the larvae at various time intervals. Almost all the first instar larvae when fed a mixture of the two viruses died from mixed virus infections, whereas in the fourth and fifth instars, most of the infected larvae showed the formation of either the tetragonal or hexagonal polyhedra and the remaining few larvae formed both polyhedra. The majority of the midgut cells of the larvae dead from mixed virus infections contained only one type of polyhedra, either hexagonal or tetragonal. Of the remaining midgut cells, a few contained polyhedra of intermediated shape and a very few contained hexagonal, tetragonal and intermediate polyhedra. It may be that a cell which is infected with one type of virus generally becomes insusceptible to the other type of virus in a short time after the initial infection. It is suggested that intermediate polyhedra may be formed as the result of random association of the tow kinds of virus particles.

**Aruga H and Hukuhara T (1961). Interference between an induced and an inoculated viruses of the mid-gut polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:31-35.**

A study was made to determine if the cytoplasmic polyhedrosis virus induced by cold treatment would interfere with the inoculated virus in the silkworm, *Bombyx mori*. It was found

that when fifth instar larvae were fed tetragonal polyhedron virus within 6h after cold treatment, almost all the infected larvae formed only tetragonal polyhedra in the midgut cells, whereas some of the larvae fed tetragonal polyhedron virus more than 12 h later from cold treatment formed hexagonal or both types of polyhedra. These results indicate that the cytoplasmic polyhedrosis virus is pre-existent in the silkworm in an occult state in which it does not interfere with the inoculated virus and that if the occult virus is induced by cold treatment, its state may be changed to an actively multiplying one in which case the virus can interfere with the inoculated different virus.

**Aruga H and Watanabe H (1961). Difference in induction rate of polyhedroses by some treatments between inbred lines and their hybrids in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:36-42.**

In the previous paper, authors reported that F1 hybrids show extended heterosis for resistance to polyhedroses in the silkworm, but when the 5$^{th}$ instar larvae, immediately after ecdysis, are exposed to low temp. (5°C) for 24 h before first feeding, the frequency of nuclear polyhedrosis is higher in F1 hybrids than in inbred lines, and the frequency of cytoplasmic polyhedrosis of F1 hybrids is apt to be in medium frequency of parental lines. In this paper, authors described the results experiment carried out to make clear if there is a correlation between polyhedroses frequencies in no treatment and in low temperature treatment or not, and to know differences in frequencies of induced polyhedrosis by high temperature treatment or by feeding of EDTA (ethylene diamine tetraacetic acid) between F1 hybrids and their parental inbred lines. The results obtained in this experiment are summarized as follows:

Among various inbred lines, there was no universal correlation between frequencies of polyhedroses in no treatment and those in low temperature treatment, ie, in resistant inbred lines to polyhedroses, induction rate of polyhedroses by low temp treatment was not always larger or smaller than that of susceptible lines. Exposing the fifth instar larvae, immediately after ecdysis, to high temp (50°C) for 30 min, polyhedroses larvae were induced in high frequency. In this case, induction rate of nuclear polyhedrosis in F1 hybrids was higher than that of parental inbred lines, whereas the rate of cytoplasmic polyhedrosis of F1 hybrids was in medium rate of parents. Thus the tendency of polyhedroses induction by high temp treatment was very similar to that of induction by low temp treatment. On the contrary to those phenomena, in the experiment of polyhedroses induction by feeding of mulberry leaves smeared with 0.5 M EDTA several times to the fifth instar larvae, frequencies of both induced nuclear and cytoplasmic polyhedroses in F1 hybrids wee smaller than those of parental lines.

**Aoki J (1961). Studies on the infection mechanism of *Aspergillus* disease in silkworm larvae, *Bombyx mori* (II) The invading loci of causal fungus. J. Sericult. Sci. Jpn. 30:43-48.**

Some investigations were conducted at first to know the parts of the body surface through which *Aspergillus flavus-oryzae* group invades the silkworm body and then to know whether symptoms of the disease are different according to the parts invaded by the causal fungus or not. Throughout each instar the invasion occurred most frequently at anus followed by inter-segmental membrane, the ratio of invasion at both loci being similar in earlier stages of larvae and incomparably larger in anus in the later stages.

Conferning whether the position of the inter-segmental membrane invaded by the causal fungus is in the anterior part of the body or not, it became clear that the fungus invades the membrane in the anterior part as well as in the posterior one in the earlier stages and the invasion is however, gradually limited to the membrane in the posterior part in the later stages. The causal fungus applicated to the inter-segmental membrane caused the shriveling of body after the next molting and the application of fungus to the anal part resulted in the constipation. Moreover,

application of fungus to both the inter-segmental membrane and anal pat caused necrosis in each part.

**Tanaka S and Nakazima F (1961). Studies on the polyhedroses of *Antheraea yamamai* and *Antheraea pernyi* (II) Induction of polyhedroses by the temperature treatment in *Antheraea pernyi*. J. Sericult. Sci. Jpn. 30:49-53. [Japanese]**

**Ayuzawa C (1961). On the induction of polyhedroses by the exposure to low temperature in the silkworm *Bombyx mori* L. J. Sericult. Sci. Jpn. 30:101-108.**

This paper concerns with the induction experiments of silkworm polyhedroses by the exposure to low temp – at 5°C for 24 h. Through the treatment, mainly nuclear polyhedrosis appeared and the occurrence of cytoplasmic polyhedrosis was very scarcely observed in this experiment. Even by successive low temp treatments in each instar soon after ecdysis, accumulative effect of treatments was not recognized, and there appeared usually one or scarcely two peaks in the distribution curve of polyhedrosis. The rate of induction in the larvae once fed with mulberry leaves after ecdysis was less than that in the larvae, which were not previously fed. When the larvae were starved for some period, even 5 h after the treatment, the rate of induction decreased. No induction was observed by the treatment during moulting period of all stages. From these results, it is considered that the rate of induction by the exposure to low temperature might be influenced by the physiological factors of silkworm before and after the treatment.

**Ayuzawa C (1961). On the possibility of screening test by the exposure to low temperature for the resistance to polyhedroses in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:109-114.**

In order to know the relationship between the rate of induction of silkworm polyhedroses by the exposure to low temperature and the resistance in natural rearing condition, the author carried out the induction experiment using a number of silkworm strains during 1957-59. Based on the rate of induction of nuclear polyhedrosis, those strains were grouped into either the resistant or susceptible strains. When the rate of induction was compared with the resistance shown in natural rearing condition, the following facts were noticed. A) The resistant strains against 5°C, 24 h treatment almost exhibited the resistance in natural rearing condition. B) The susceptible strains against the low temp treatment did not always exhibit the susceptibility in natural rearing condition. Accordingly, the method of exposure to low temp was considered not to be always applicable for the screening test in the breeding of the resistant strains of the silkworm against the polyhedrosis.

**Ayuzawa C (1961). On induction of nuclear polyhedroses by the intermittent exposure to low temperature in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:115-118.**

The effect of intermittent short exposure to low temp upon the rate of silkworm nuclear polyhedrosis was examined. The larvae were exposed to low temp intermittently twice or thrice at 5°C, for 2-6 h and were kept at 25°C at interrupting period. The rate of occurrence of polyhedrosis was nearly the same as that of the continuous exposure to low temp when the period of interrupted exposure at 25°C was shorter that 1 h. However, the rate of induction decreased when the period of interrupted exposure was longer than 2 h.

**Aruga H and Israngkul A (1961). Studies on the size of cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:119-125.**

A few experiments were carried out on such factors controlling the size of cytoplasmic polyhedra of the silkworm, *Bombyx mori*, as the root of the occurrence of cytoplasmic

polyhedrosis (inoculation or induction), midgut portion, larval instar, incubation period, and latent infection. The polyhedra formed in the anterior portion of mid-gut were larger than those in the posterior one in the case of inoculation, but in the diseased larvae induced by cold treatment, no such difference in the size of the polyhedra occurred throughout the diseased midgut. The size of polyhedra formed in the larvae which had been inoculated in one of the larval instars and died on the 7$^{th}$ day after the feeding of polyhedra was nearly the same. The observation of diseased larvae at various time intervals after inoculation revealed that the longer the time interval the larger the polyhedra formed. However, if newly hatched larvae were fed very small amount of virus and observed in different instars, 3$^{rd}$ instar larvae contained somewhat larger polyhedra than 4$^{th}$ and 5$^{th}$ instar larvae. From this it may be plausibly thought that some of the inoculated larvae began to develop the disease immediately after inoculation but some others retain the viruses in a latent (or occult) state to the later instars.

**Tanaka S (1961). Studies on the polyhedroses of *Antheraea yamamai* and *Antheraea pernyi* (III) On the relation between the rearing season and the polyhedrosis in *Antheraea pernyi*. J. Sericult. Sci. Jpn. 30:126-130. [Japanese]**

**Nunome J, Yawata Y and Matsubara F (1961). Studies on air hygiene of silkworms. III. Relations between frequency of litter cleaning of ordinarily reared silkworms in later stage and pH values of silkworm blood and falling ill. J. Sericult. Sci. Jpn. 30:135-139.**

Decreasing in frequency of litter cleaning in later stage of ordinarily reared silkworms, silkworms showed signs of acidiosis and fall ill (most diseases occurred in this study were grasseries). And in the scope of this study, the frequency of litter cleaning is proportional to pH values of silkworm blood and inversely proportional to the rate of falling ill. Consequently, it may be possible to know the relation between the frequency of litter cleaning and health condition of the silkworm to some extent by investigating pH values of silkworm blood.

**Ishikawa Y and Asayama T (1961). Studies on the relation between the polyhedroses of the wild insects and the silkworm, *Bombyx mori* L. (III) On the double infection of the nuclear polyhedroses in silkworm larva. *J. Sericult. Sci. Jpn*. 30:201-205.**

This paper describes about the double infection of two kinds of two nuclear polyhedroses in the silkworm larva. A double infection of the nuclear polyhedroses in silkworm larvae was first found in some of the matured larvae, which were, on the first day of the 5$^{th}$ instar, infected with the polyhedral virus of *Philosamia Cynthia pryeri* B. In the tissues infected with both viruses, there were formed polyhedral bodies of *B. mori* and *P. pryeri*. But in each nucleus, only one kind of polyhedral body was always formed and were not both kinds of polyhedral bodies. In a very few cases, it was observed that large and triangular polyhedra, which were different from the other two polyhedra in shape and size, were formed in the nuclei of the infected tissues.

**Aruga H and Hukuhara T (1961). Latent infection in the cytoplasmic polyhedrosis of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:334-338.**

A study was made to determine whether latent infection with the cytoplasmic polyhedrosis virus took place during the rearing of the silkworm. Silkworm eggs were smeared with different concentrations of tetragonal cytoplasmic polyhedrosis, but the others grew normally. Upon reaching the fifth instar, some larvae were refrigerated for 24 h and the others untreated ones were kept as controls. Observations of the occurrence of different types of cytoplasmic polyhedrosis in these 5$^{th}$ instar larvae revealed that the proportion of infected larvae containing tetragonal polyhedra was higher in the groups of larvae which had hatched from the eggs smeared with relatively high concentrations of polyhedra than in the control groups (no virus smeared). On the other hand, the effect of the treatment upon the eggs was not so conspicuous in the groups of larvae having hatched from the eggs smeared with relatively low concentrations of

polyhedra. The results that most of the infected larvae which appeared in these groups and in the control groups contained hexagonal polyhedra suggested that the hexagonal polyhedron virus in an occult state might be transmitted from the previous generation. It was proposed that some of the larvae which were fed with relatively high concentration of virus at the time of hatching retained the virus until the beginning of the fifth instar in such a state that it neither formed polyhedra, nor interfered with a later inoculated virus, nor was detectable as an infective virus.

**Ishikawa Y and Asayama T (1961). On the amount of cytoplasmic polyhedra which causes infection in the larvae of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:339-344.**

In this paper, authors described the relationship between the virulence of Bm cytoplasmic polyhedra and the degree of susceptibility to the *Bombyx mori* larvae.

**Hukuhara T (1961). Induction of cytoplasmic polyhedrosis by EDTA in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:351-353.**

In a previous paper, it was reported that ethylene di-amine tetra-acetic acid (EDTA) and its di-sodium salt (Na-EDTA) could induce the cytoplasmic polyhedrosis of the silkworm. In the present study the two chemical were fed to silkworm larvae in different instars. It was found that EDTA was more effective than Na-EDTA in the induction of cytoplasmic polyhedrosis. The aptitude of silkworm larvae remained so low from the first to the 3$^{rd}$ instar that cytoplasmic polyhedrosis was hardly induced, whereas it became higher in the 4$^{th}$ instar and reached a very high level in the 5$^{th}$ instar. The most effective way to induce cytoplasmic polyhedrosis was to feed 0.1 M concentration of EDTA to the 5$^{th}$ instar larvae.

**Aizawa K, Furuta Y and Nakamura K (1961). Selection of a resistant strain to virus induction in silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:405-412.**

The experiment concerns with the selection of a silkworm resistant to virus induction. Larvae of a silkworm strain were reared as usual, except for treatment by refrigeration at 5°C for first 24 h after the ecdysis of the 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ instar respectively and eggs were obtained from the survived moths. The diseases appearing by this experiment were nuclear and cytoplasmic polyhedroses and flacherie. This treatment was performed through 13 generations, and in the 11$^{th}$ to 13$^{th}$ generation one batch rearing was made. Survival rate of a selected strain from these generations was always higher than that of the original (non-selected ) strain and the resistance to virus induction by means of cold treatment was recognized.

Subsequently, the effect of malnutrion was compared with selected and non selected strains. The larvae were reared on harder mulberry leaves during the 1$^{st}$ and 3$^{rd}$ instars and on rotten leaves during the 4$^{th}$ and 5$^{th}$ instars. In the common strain C 115 x J 122 all larvae died by the 5$^{th}$ instar and the non-selected strain 4 out of 413 larvae pupated, but eventually died, while in the selected strain 130 out of 325 larvae became adult. From this result, it was considered that the selected strain by means of cold treatment was resistant to virus induction by malnutrition.

Since the selection has been performed under condition of mass-rearing, a question arises whether the larvae of selected strain will be reinfected with the infectious virus from outside, by which a lysogenization like phenomenon will be caused. For this purpose, the nuclear polyhedrosis virus was injected into the silkworm pupae and the eggs laid by the heavily infected moths were obtained. Even with such eggs, it was not possible to find out distinct difference in the induction rate by the exposure to cold treatment or at normal rearing between virus injected and non injected batches and it was considered that a lysogenization-like phenomenon would not appear.

The resistance to virus infection was examined employing the selected strain by means of cold treatment, non-selected strain and other silkworm strains. The difference in the susceptibility (log $LD_{50}$) for the nuclear polyhedrosis virus was not remarkable among them, and thus the resistance to virus induction seems to be independent from that to virus infection.

**Ayuzawa C (1961). On the resistance to infection of nuclear polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 30:413-419.**

This paper deals with the resistance in the infection of the nuclear polyhedrosis virus by means of REED & MUENCH'S method and the latent period in larvae and pupae employing several silkworm strains. The polyhedra were dissolved in alkaline solution and inoculation was carried out with sterilized glass capillaries. Regulating the hatching, inoculation was done for the larvae and pupae at the same stage in each experiment. The results obtained are as follows:

No difference in $LD_{50}$ between male and female was observed. A marked difference in $LD_{50}$ was not recognized between the 4$^{th}$ moulting period (resistant to induction) and the early period of the 5$^{th}$ instar 5-7 h after the 4$^{th}$ ecdysis (sensitive to induction). No remarkable change of the resistance to infection was observed along the development, in comparison with the resistance to induction by the low temperature treatment, which is remarkably different between before and after ecdysis. Difference over 2.00 in $LD_{50}$ among the strains were not recognized and strains resistant to the induction were not always resistant to the infection. Latent period in pupae were different by silkworm strains and strain was shown to be one of factors controlling the length of the latent period.

## Vol. 31 1962

**Aruga H and Yoshitake N (1962). Further studies of interference between two viruses of the midgut polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 31:11-16.**

The authors investigated how the interference between tetragonal (T) and hexagonal (H) cytoplasmic polyhedron viruses in the silkworm was modified by such conditions as the state of the viruses, physiological condition of host larva, and relative concentration of the two viruses.

The interference between the two viruses when the 5$^{th}$ instar larvae had been starved for 48 h after ecdysis was very similar to the interference observed in the case of non-starvation. When the larvae inoculated with tetragonal polyhedron suspension at the 4$^{th}$ instar stage just after ecdysis were treated by cold at the 5$^{th}$ instar just after ecdysis, polyhedrosis with tetragonal polyhedra occurred at higher frequency than in the case of non cold treatment, showing the fact that the higher the concentration of inoculated virus, the higher was the occurrence of polyhedrosis. Whereas it was recognized that the lower the concentration of tetragonal polyhedron suspension the higher was the occurrence of polyhedrosis with hexagonal cytoplasmic polyhedra or nuclear polyhedron. Such relation was not recognized in the experiment in which the larvae had been inoculated with tetragonal polyhedron suspension in the first instar just after hatching and treated by cold at the fifth instar just after ecdysis. Such phenomenon might be due to the fact that there was some difference in the state of latently infected viruses contained in larvae of the 5$^{th}$ instar between the 1$^{st}$ and the 4$^{th}$ instars inoculations of interfering viruses. Experiments have been carried out on the interference between the two different kinds of virus in several combinations of virus concentration. It was clarified that the manifestation of interference was modified by several combinations of low or high concentration in both the interfering and challenging viruses.

It was ascertained that in a few cases the primary inoculated virus which was plausibly thought to be either in a non-multiplying or scarcely multiplying state in larval body could interfere with the multiplication of the challenge virus. It was pointed out that the prevention of

the occurrence of cytoplasmic polyhedrosis in the silkworm would be possible by utilizing the above mentioned phenomena.

**Aruga H, Yoshitake N and Watanabe H (1962). Interaction between inactivated virus and activated virus of midgut polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 31:17-24.**

The present paper deals with the interference between inactivated and challenge viruses of cytoplasmic polyhedrosis in the silkworm. Cytoplasmic polyhedron virus (5% polyhedron suspension) in the silkworm is inactivated by the treatment of 80°C for 10-15 min or 60°C for 140-160 min. The occurrence of cytoplasmic polyhedrosis caused by challenge virus can be prevented in some degree by inoculating silkworm larvae with the inactivated virus. Consideration has been made on the mechanism of inhibition of reproduction of challenge virus by the inactivated virus.

**Yamaguchi S (1962). Studies on the functional localization of digestive system in the sw larva, *Bombyx mori* L. (VI) Susceptibility and its local difference of the midgut epithelial cell for virus multiplication. *J. Sericult. Sci. Jpn*. 31:90-96.**

The histo-pathological investigation was undertaken in an attempt to know the susceptibility and its local difference of the midgut cells of silkworm larva, on which the treatment such as (1) refrigeration, (2) virus feeding and (3) virus feeding after refrigeration were performed immediately after the 4$^{th}$ ecdysis. The results were summarized as follows:

When the silkworm larvae are refrigerated, the larvae fall into gattine like disease, and the cytoplasm of the midgut cylindrical cell become to have many vacuoles and the nuclei begin to swell up remarkably in several days. It is interesting to see that the similar figures are observed in the stud of gattine by PAILLOT (1930). In the experiment of virus feeding, the cytoplasmic polyhedron did not appear, but the similar histo-pathological changes mentioned above were also observed. In the case of combined treatment with virus feeding and refrigeration, the cytoplasmic polyhedron appeared. The changes seen in the experiments 1 and 2 were not seen in nucleus, though the degeneration and disappearance of nuclei were often noticed after completion of polyhedron formation. The histo-pathological changes in cytoplasm and nucleus are usually observed at first in the posterior division of the midgut epithelium and then appear in middle and anterior ones. It may be said that the cell susceptibility to the pathogenic injuries differs according to the treatments and the division of midgut epithelium, and in the latter case the susceptibility of the cells are the most intensive in the posterior division compared with the cells of the middle and anterior divisions.

**Hukuhara T (1962). Transmission of the cytoplasmic polyhedrosis virus from one generation to the next generation in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 31:97-100.**

5$^{th}$ instar silkworm larvae just after ecdysis were inoculated with either hexagonal or tetragonal cytoplasmic polyhedron virus. Some of the inoculated larvae died during the larval stage, but some emerged as adults. They were crossed between each other. When the larvae hatching from the eggs which were obtained from these crosses reached the fifth instar, cold treatment was carried out on them to induce the development of cytoplasmic polyhedrosis. When larvae died after the cold treatment, they were examined microscopically. The proportion of the number of larvae containing tetragonal cytoplasmic polyhedra in the midgut as compared to the number dead from cytoplasmic polyhedrosis was higher in the progeny of silkworm which were inoculated with hexagonal polyhedron virus. The author is inclined to postulate that normal silkworms transmit the cytoplasmic polyhedrosis virus of the hexagonal type to the next generation in an occult state through the germ cell and that when normal larvae were inoculated

with the tetragonal polyhedron virus, some of the larvae transmit the tetragonal polyhedron virus to a part of the next generation in an occult state.

**Aruga H and Nagashima E (1962). Role of chromosomes and cytoplasm for the resistance to cytoplasmic polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 31:101-107.**

To clarify the mechanism of resistance to polyhedroses in silkworm, the relation between the frequency of cytoplasmic polyhedrosis and chromosome constitution (sex chromosomes and autosomes) in several hybrids has been studied genetically. The original races which had been used for hybridization were E16, N124, C124 and Cambodge. The frequency of cytoplasmic polyhedrosis was high in E16 and N124, but was low in C124 and very low or non in Cambodge. The hybrids analyzed in the present experiments were back cross because of the fact that back crossing was thought to be good for analysis on the role of chromosome constitution for resistance to polyhedrosis in *B. mori* from the genetical point of view. The occurrence of cytoplasmic polyhedrosis in the 5$^{th}$ instar larvae of those hybrids was investigated under the both ordinary rearing condition and exposure to low temperature (5°C, 24 h) just after the 4$^{th}$ ecdysis. The results obtained are as follows.

Cytoplasmic polyhedrosis was scarcely observed under the ordinary rearing, but considerable numbers of cytoplasmic polyhedrosis were observed in the cold treatment. The result that sex chromosomes (X and Y) and autosomes played important parts for the resistance to the disease was not recognized in several hybrids.

From the above mentioned results and the phenomenon that the virus which had been inoculated to the first instar larva of previous generation might be plausibly thought to be transmitted from the female moth to the 5$^{th}$ instar larva of next generation through eggs in a latent state (ARUGA and NAGASHIMA, 1962, unpublished), it seems likely that the occurrence of cytoplasmic polyhedrosis in *B. mori* is markedly influenced by the cytoplasmic condition which are controlled by both the cytoplasmic characteristics in egg affected by female moth and the chromosome constitution.

**Yamaguchi S (1962). Studies on the functional localization of digestive system in the silkworm larva, *Bombyx mori* L. (VII) Distribution and behaviour of nucleic acid in the midgut cells of the silkworm larva in polyhedrosis. *J. Sericult. Sci. Jpn*. 31:114-121.**

Distribution behavior of nucleic acids in the virus diseased cells were investigated on the midgut epithelium of silkworm larva, *Bombyx mori* L.

From the observations by means of suravital staining and histo-chemical method, it was ascertained that in the feeding time of the healthy larva RNA is generally distributed in the cytoplasm of cylindrical cell of the midgut, and DNA is generally rich and is constant in quantity. It was observed that RNA is contained abundantly in several nucleoli in some of the nucleus of cylindrical cell. Among the heavily virus diseased cells the degenerated nuclei, which are narrow and irregular in shape, are sometimes observed and they are stained pale or remain unstained. This fact seems to show that the nucleus of diseased cell is deficient in DNA. In the newly hatched larvae and in the larvae shortly after ecdysis, RNA is poor in quantity in midgut cells, and increases in quantity after feeding. From the facts that RNA contents in the nucleus of cylindrical cell usually increase prior to the increase of the cytoplasmic RNA contents, it may be considered that there are close relation between the behaviours of RNA both in nucleus and cytoplasm. In the early stage of cytoplasmic polyhedrosis the cytoplasm of cylindrical cells begins to contain a large quantity of RNA, especially on the outside of nuclear membrane, on the top of the cell and around the forming polyhedra. However, RNA decreases after completion of

polyhedra formation. RNA is contained abundantly in the posterior division of the midgut epithelium in the feeding larva, less in the anterior one and the least in the middle.

**Suzuki C, Karasawa T and Kimura R (1962). Relation between the occurrence of silkworm disease and the three factors, air current, air composition and temperature. *J. Sericult. Sci. Jpn*. 31:134-138.**

By means of simultaneous combination, the effects of three factors, air current, air composition and temperature upon the attack rate of silkworm disease, flacherie and grasserie, were researched. Air composition and temperature affect directly upon the attack rate of the disease, mean while the air current affects indirectly. Even in later instar, the effect of high humidity upon the attack rate seems to be not so great as that become sensational. From 80 to 97% of all diseased silkworm were flacherie F-type. An application of the results was tried to illustrate the so called "Zamure" – a musty state of micro-climate in the silkworm rearing seat which is seemed to be a cause of the diseases.

**Aruga H, Yoshitake N and Watanabe H (1962). The induction of polyhedroses by treating with low and high temperature in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 31:139-142.**

The induction of polyhedroses in the silkworm was investigated treating the 5$^{th}$ instar larvae just after ecdysis with low temp – 5°C, 24 h and high temp (hot water bath at 40°C for 5 min or dry heat shock at 50°C for 30 min. The frequency of cytoplasmic polyhedrosis induced by cold and successive heat treatment was clearly less than that induced by cold treatment alone. But if the larvae treated with cold were left at room temp (25°C) for 30 – 120 min till the next heat treatment, the frequency of induced cytoplasmic polyhedrosis was less than or about the same as that in the case of cold treatment alone. On the frequency of the induction of nuclear polyhedrosis, no such clear difference was observed between cold and heat treatment and cold treatment alone. In the combined cold and heat treatment, the above mentioned results were obtained irrespective of order of temperature treatment, and hot-water and dry heat affected similarly as the stressor.

**Aoki J (1962). Studies on the infection mechanism of *Aspergillus* disease in silkworm larvae, *Bombyx mori*. (III) The relationship between development of the causal fungus, *Aspergillus flavus-oryzae*, and its attack to larvae under different temperature conditions. *J. Sericult. Sci. Jpn*. 31:143-148.**

In the present paper, the relation was made clear between the temperature condition and the vitality of *Aspergillus flavus-oryzae*, the causal fungus of *Aspergillus* disease in silkworm, together with the influence of rearing temperature on the attack of this disease.

When the 1$^{st}$ and 2$^{nd}$ instar larvae, reared at 20, 25 and 30°C were inoculated by the causal fungus, temperature rise augmented the attack, accompanied also by the quicker formation of conidiospores in potato decoction-agar medium with 2% sucrose and by the promotion both in spore germination and mycelial growth in CZAPEK'S solution. In addition, the larvae were highly susceptible to the invasion even by low concentration of fungus solution in contrast to the extreme resistance to invasion of the 4$^{th}$ instar larvae. It can be substantiated, from above results, that the high temperature rearing of the younger silkworm, the 1$^{st}$ and 2$^{nd}$ instar is highly dangerous to the invasion of the causal fungus.

**Tanaka S (1962). Studies on the polyhedroses of *Antheraea yamamai* and *Antheraea pernyi*. (IV) On the size of nuclear polyhedra in *Antheraea pernyi*. *J. Sericult. Sci. Jpn*. 31:149-153.**

In this report the author deals with the size and its variation of nuclear polyhedra formed in larvae of several stages and pupae in *Antheraea pernyi*. The size of polyhedra contained in the blood of diseased pupae were larger than that observed in the blood of diseased larvae, and the polyhedra produced in the larvae of early developmental stages were smaller than those formed in the larvae of later stages.

The mean size of 500 polyhedra in five individuals (100 polyhedra were measured in one individual) was 1.64±0.35 μm on the 12$^{th}$ day in the 5$^{th}$ instar larvae, and was 2.48±1.09 μm in pupae, being 7.56 μm as the largest size of polyhedron in pupal stage. And the range of the variation of size of polyhedra in pupa was higher than in larva.

Virus infection was carried out by using of polyhedral virus from larvae to pupae and from pupae to larvae reciprocally. The results showed that the size of polyhedra was also larger in pupa than in larva. In this experiment a large polyhedron which was not observed in larva was recognized in pupa. From the above mentioned results it may be plausibly thought that the remarkable fluctuation of the size of polyhedra formed in pupae was not caused by the difference of virus strains, but was controlled by the difference of host cells which differentiate from larval stage to pupal one.

**Aoki J (1962). Studies on the infection mechanism of *Aspergillus* disease in silkworm larvae, *Bombyx mori*. (IV) The pathogenicity of the causal fungus, *Aspergillus flavus-oryzae*, in relation to pigment production, protease activity and amylase activity. *J. Sericult. Sci. Jpn*. 31:221-227.**

The difference of pathogenicity for silkworm was investigated in connection with the pigment production and the activity of protease and amylase in 27 strains of *Aspergillus flavus-oryzae*. Inoculating the conidiospore suspension of these causal fungi into newly hatched sw larvae, the rate of attack till the end of the 2$^{nd}$ molting was examined and it was made clear that the pathogenicity was different by each strain, eg, over 70% in 8 strains and under 20% in 6 strains. The pigment production was more active in stronger pathogenic strains, when cultured for 30 days at 30°C on slant agar media. The activity of amylase in terms of saccharogenesis and dextrinogenesis increased in weaker pathogenic strains. The ability of gelatin dissolution and protease activity augmented in stronger pathogenic strains.

**Aizawa K and Furuta Y (1962). Resistance to virus induction in F1 hybrids between resistant and common strains in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 31:245-252.**

The selection of a silkworm strain resistant to virus induction was done by means of cold treatment through 13 generations (AIZAWA *et.al.*, JSSJ 30: 405-412). Further selection has been performed and the survival rate of this strain was always higher than that of the original (non-selected) strain in the 14$^{th}$ to 16$^{th}$ generation when larvae were exposed to low temp (5°C to 24 h) after ecdysis of the 4$^{th}$ and 5$^{th}$ instars. It was also shown that this strain was resistant to virus induction by means of malnutrition as reported previously.

In F1 hybrids between the strain resistant to virus induction and a common strain (J124), the survival rate was higher than those of their parents and the induction rate of polyhedroses was lower than those of their parents in late autumn 1961. However, a quite opposite result was obtained in spring 1961. The quality of cocoon in F1 hybrids was better than that of their parents.

**Aizawa K, Kawarabata T and Sato F (1962). Response of the silkworm, *Bombyx mori*, to *Bacillus thuringiensis* BERLINER. *J. Sericult. Sci. Jpn*. 31:253-257.**

The $LD_{50}$ of both Bactospeine and Thuricide WP were measured using three silkworm strains (C108 x J115, J124 x C124 and C115 x J122) and C108 x J115 was found to be most resistant to toxicity of these bacterial insecticides among three strains. Spores of *B. t.* were killed by boiling and by 2% HCHO (gas contact), further the toxicity of bacterial insecticide for the silkworm was lost by these treatments employing Bactospeine. Spores were killed by 0.1% $HgCl_2$, however, the toxicity was still retained. In two larvae out of ten larvae examined, multiplication of *Bacillus thuringiensis* in dead larvae was recognized and crystal toxins were not found in dead larvae. On the contrary, *B.t.* multiplied by injection of spores into pupal body, pores and crystals toxins were easily found in dead pupa.

**Suzuki C, Kimura R and Suzuki K (1962). Effect of microclimatic environment upon the attack-rate of cytoplasmic polyhedrosis in the inoculated silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 31:329-334.**

Relation between microclimatic environment around silkworm larvae and occurrence of the disease in the larvae when they were inoculated *per os* with tetragonal cytoplasmic polyhedra (Ct) was investigated. Under some unsuitable environments there occurred several kinds of diseases. When the environment was unsuitable for the larvae, increase in total attack rate of the disease in those inoculated with a small quantity of Ct was larger than that of non-inoculated ones, while no difference was found between them when the environment was quite suitable. Ratio of attack rate of Ct to total one of victims of related diseases became larger with the total attack rate.

When the dose of Ct in the suspension for inoculation was exactly small, the latent period of Ct in larvae under a suitable environment was longer than that under a unsuitable one. It is supposed that the occurrence of pathogenic induction of cytoplasmic polyhedrosis may, if ever, be hardly happened under severe incubating or rearing conditions or cold treatment, and that the infection will play an important role in disease induction of the cytoplasmic polyhedrosis.

**Aoki J (1962). Further studies on invasion to the wooden parts of rearing tools by *Aspergillus flavus-oryzae* group. *J. Sericult. Sci. Jpn*. 31:378-382. [Japanese]**

## Vol. 32 1963

**Aruga H, Yoshitake N and Owada M (1963). Factors affecting the infection *per os* in the in the cytoplasmic polyhedrosis of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:41-50.**

Effects of several stressors were studied on sw larvae to which the CPV was administered *per os* before or after the stress. Among stressors tested those which increased the incidence of cytoplasmic polyhedrosis were cold (5°C for 3~5 h), heat (37°c for 5 h), formalin (0.01~1%), EDTA (powder) and acetic acid (0.1~0.5 M). On the other hand, calcium hydroxide (powder) decreased the incidence of the disease. The mechanism of the increase in the incidence of the disease may possible be: (1) the stressors make the penetration of the virus easier, (2) the stressors accelerate the multiplication of the virus in infected cells, and (3) the stressors induce the development of the disease. It was discussed that the first two explanations were more probable.

**Aruga H, Watanabe H and Nagano H (1963). Interference by the heat-inactivated virus on the active virus of the cytoplasmic polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:51-57.**

In the present study the interference of the active virus by the heat-inactivated virus of the cytoplasmic polyhedrosis in the silkworm has been established. The virus contained within the

purified polyhedra that were free of insect tissues and the free virus obtained from polyhedra dissolved with 0.5-5% $Na_2CO_3$ solution were inactivated by heat treatment at 85°C for 15 min. When larvae were fed with either the inactivated polyhedron virus or the inactivated free virus, the development of cytoplasmic polyhedrosis caused by the active virus which was administered subsequently was generally reduced. The inactivated free virus was much more effective in subsequently was generally reduced. The inactivated free virus was much more effective in preventing infection by the challenge virus than the inactivated polyhedron virus. The results of the interference by the inactivated free virus on the active viruses of the homologous and heterologous strains of cytoplasmic polyhedrosis, ie, the strains that formed tetragonal and hexagonal polyhedra, revealed that the degree of interference on the homologous strain was similar to that of the heterologous strain, but there tended to be a little greater interference in the latter.

**Aruga H, Yoshitake N, Hukuhara T and Owada M (1963) Invasion route of the cytoplasmic polyhedrosis virus in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:58-62.**

A study was made to know through which part of the midgut the cytoplasmic polyhedrosis virus was able to penetrate in the fifth instar larva of the silkworm. In general, after microfeeding of the virus, the larva whose midgut cells had been injured by ligature with silk thread for an hour contained polyhedra in the midgut posterior to the ligature when the ligature was applied behind the sixth or seventh segment. When the ligature, however, was applied behind the eighth or ninth segment, all portions of the midgut were infected. These results indicated that the ingested virus penetrated into the cells of the midgut in a region posterior to the seventh segment and the virus then spread from cell to cell to the cephalic portion of the midgut. Virus propagation appeared to be inhibited at the region where the cells were injured. When the virus was inoculated subcutaneously, it seemed to initiate infection anywhere in the midgut. The polyhedra formed in the cephalic portion of the midgut were larger than those formed in the caudal portion.

**Aruga H, Fukuda S and Yoshitake N (1963). Observations on a polyhedrosis virus within the nucleus of the silk gland cell of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:213-218.**

Virus polyhedra within the nuclei of silk gland cells occurred in nearly all of the 3$^{rd}$, 4$^{th}$ and 5$^{th}$ instar larvae, which were infected with nuclear polyhedrosis. Larvae in the younger instars, however, had no polyhedra within their silk gland cells. In the silk gland cells, there were four to eighteen virus rods within one developmental membrane, while in the hypodermal cells, there were fewer number, one to four rods within one membrane. There was no difference between the larvae of the mutant silkworm strain (*Nd*) and those of the normal strains in the formation of polyhedra within the silk gland cells.

**Ayuzawa C (1963). The occurrence of nuclear polyhedrosis by the exposure to a low temperature in the virus inoculated larvae of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:219-225.**

The paper deals with the occurrence of nuclear polyhedrosis of the silkworm by the exposure to a low temp (at 5°C for 24 h) at the 5$^{th}$ instar, with survived larvae which had been inoculated (*per os*) with the virus at younger stages. Purified nuclear polyhedra were dissolved with an alkaline solution and silkworm larvae were fed with different concentration of virus. In these experiments, no cytoplasmic polyhedrosis appeared. There was not remarkable difference in induction rates by the exposure to a low temp between the survived larvae which had been fed with the virus at younger stages and the non-inoculated larvae. However, the occurrence of this disease in the survived larvae which had been fed with polyhedra at the beginning of the 4$^{th}$ instar have the tendency of increase in the induction rate by exposure to a low temp at the 5$^{th}$ instar.

**Tanaka S and Aruga H (1963). The effect of cold treatment on the polyhedrosis virus infections in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:226-231.**

Studies on the occurrence of the nuclear and cytoplasmic polyhedroses in the silkworm were carried out by inoculating the 4$^{th}$ instar larvae or the 5$^{th}$ instar larvae shortly after ecdysis with the NPV or CPV. The larvae were inoculated with the virus prior to or after the cold treatment (5°C 24 h).

The incidence of nuclear polyhedrosis in larvae was markedly higher when the larvae were fed the nuclear polyhedrosis virus and exposed to low temp than when they were only fed the virus or only exposed to low temp. When the cytoplasmic polyhedrosis virus was fed to newly molted 5$^{th}$ instar larvae after the cold treatment, a high rate of mixed infections of both the nuclear and cytoplasmic polyhedroses were observed. It is speculated that the occurrence of a phenomenon such as the one mentioned above may be due to the induction of the nuclear polyhedrosis by the inoculation with the cytoplasmic polyhedrosis virus in association with the cold treatment.

The experimental results of the two treatments, low temp and virus inoculation showed that, when the newly molted 5$^{th}$ instar larvae were inoculated with the virus shortly after cold treatment, there were a higher incidence and an earlier appearance of polyhedroses than when the larvae were inoculated with the virus on the second day of the 4$^{th}$ instar and treated with low temp at the 5$^{th}$ instar.

**Utsumi S and Chikushi H (1963). Studies on the inhibition of polyhedroses in the silkworm, *Bombyx mori* L. (1) The effect of taurine and cystine on the incidence of polyhedroses. *J. Sericult. Sci. Jpn*. 32:232-239.**

The silkworm larvae are generally infected with polyhedroses after cold treatment, when the larvae are maintained at 5°C for 24 h just after ecdysis, or after the inoculation of the polyhedroses viruses. And the authors have found that the nuclear polyhedrosis was inhibited when the larvae were fed with mulberry leaves sprayed with taurine or cystine solutions of 5 x $10^{-2}$ mol prior to the cold treatment or after the virus inoculation. Taurine had a greater inhibitory effect than cystine. But the effects of taurine and cystine on cytoplasmic polyhedrosis were uncertain.

**Suzuki C, Kimura R and Suzuki K (1963). On the pathogenic power of flacherie diseases occurred with different causes in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 32:240-242.**

In order to know the pathogenic power of a flacherie group occurred with some cause, newly hatched silkworm larvae were administered homogenate of each paticent and reared and were observed the attack rate. As the results of the experiments, three typical types in the mode of the frequencies ie, 1) the frequencies were inclined to 10/10 attack rate, 2) to 0/10, and 3) to both side were obtained. According to the authors' experiments in their laboratory at Maebashi campus, those occurred with inoculation of flacherie virus belonged to 1 type and those with rearing under unfavourable conditions to 3 type and those occurred rarely in normal rearing to 2 type.

**Takizawa Y and Iizuka T (1963). Studies on the toxicity of some *Bacillus* upon the silkworm, *Bombyx mori* L. (I) Influence upon young larvae. *J. Sericult. Sci. Jpn*. 32:343-347.**

The susceptibility of young larvae of silkworm, against 8 species of Bacillus group (two kinds of crystalliferous bacteria and 6 kinds of acrystalliferous bacteria) was investigated. Two groups were prepared for treatments. A) eggs were directly sprayed with spore suspensions. B) mulberry leaves were sprayed with spore suspensions.

*B. thuringiensis* var. *thuringiensis* and *B. thuringiensis* var. *sotto* were toxic to sw larvae, while the toxicity was not observed with *B. cereus, B. cereus* var. *mycoides, B. megaterium, B. subtilis 1,037, B. subtilis 1,038 and B. subtilis 1,039.* 18 larvae out of 20 died after showing paralysis, when fed leaves wich were sprayed with $10^6/mm^3$ spores of *B. thuringiensis* var. *sotto.* At the conc. of $10^5/mm_3$ spores, 18 larvae out of 20 showed paralysis, however, these larvae continued growing being 48 h when compared with the growth of control group.

**Takizawa Y and Iizuka T (1963). Studies on the toxicity of some *Bacillus* upon the silkworm, *Bombyx mori* L. (II) Influence upon fifth instar larvae. *J. Sericult. Sci. Jpn*. 32:348-352.**

The susceptibility of 5th instar larvae of silkworm, against 8 species of *Bacillus* group (two kinds of crystalliferous bacteria and 6 kinds of acrystalliferous bacteria) was investigated by feeding and injection.

In the method of feeding foliage contaminated with two kinds of two kinds of crystalliferous bacteria and 6 kinds of acrystalliferous bacteria, the former was toxic to the 5th instar larvae and the latter was not toxic. Vegetative cells were observed the midgut and in the haemolymph of larvae after feeding spores of *B. thuringiensis* var. *sotto* at 24 h. When larvae fed spores of *B. thuringiensis* var. *thuringiensis*, vegetative cells were observed in the midgut and in the haemolymph just before death. Septicemia was caused by injection of spores in 8 *Bacillus species.*

## Vol. 33 1964

**Iwashita Y and Aruga H (1964). Induction of polyhedroses in larvae subjected to high temperature and humidity in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 33:124-129.**

Third and fourth instar silkworm larvae were subjected to high temp (38°C) and humidity (90%) for 48 h. On account of this treatment the molting character of the larvae changed. Some became recessive trimolting larvae, some became dominant trimoltine larvae and some remained normal tetramolting larvae. The incidence of nuclear and cytoplasmic polyhedroses was higher among the larvae subjected to the treatment in the 4th instar than among the larvae treated in the 3rd instar. When compared with the recessive trimolting or tetramolting larvae, the dominant trimolting larvae were less liable to develop polyhedroses either spontaneously or after the cold treatment (5°C for 24 h).

**Ayuzawa C and Yusa F (1964). On the inactivation of the viruses of the silkworm, *Bombyx mori* L. by the $^{60}$Co-$_\gamma$ ray irradiation. *J. Sericult. Sci. Jpn*. 33:130-133.**

This paper deals with the inactivation of the nuclear and cytoplasmic polyhedrosis viruses and infectious flacherie virus of the silkworm, *Bombyx mori* L. with the irradiation (within 50.0 Kr dose) of $^{60}$Co-$_\gamma$ ray. $LD_{50}$ (-log) values were calculated according to the BEHRENS-KÄRBER's method.

Nuclear-polyhedrosis virus: Purified and dried polyhedra were employed. Virus titer was determined by means of the injection to pupae of the virus solution, dissolved in alkaline solution. The $LD_{50}$ values show that the virus is inactivated only very slowly. Cytoplasmic polyhedrosis virus: Suspension of purified polyhedra was employed. Virus titer was determined by means of the *per os* infection of the newly hatched larvae. The $LD_{50}$ values show that the virus is not inactivated. Infectious flacherie virus: The filtrate through Seitz EK of the 10% homogenate of infected larvae was employed. Virus titer was determined with the *per os* infection of the newly hatched larvae. The $LD_{50}$ values show that the virus is hardly inactivated.

**Watanabe H (1964). Temperature effects on the manifestation of susceptibility to peroral infection with cytoplasmic polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 33:286-292. [English]**

In the present study, the author studied the effect of a slight raising or lowering of the temperature from the normal upon the infection with the cytoplasmic polyhedrosis virus when the silkworm larvae were reared at these temperature for several days before infection. He also investigated the manifestation of heterosis on the resistance to virus infection when the larvae were stressed by the abnormal temperatures. The present paper deals largely with these two aspects.

**Tanaka S (1964). Effect of hormonal action on the inefectivity with nuclear-polyhedrosis viruses in the pupae of *Samia cynthia pryeri* and *Antheraea pernyi*. *J. Sericult. Sci. Jpn.* 33:317-320 [Japanese]**

**Aruga H and Yoshitake N (1964). Interaction between cytoplasmic-polyhedrosis viruses in *Bombyx mori* (LINNAEUS) under certain conditions. *J. Sericult. Sci. Jpn*. 33:345-351. [English]**

In the silkworm, *Bombyx mori* L., interference occurs between the two types of cytoplasmic-polyhedrosis viruses which produce icosahedron and hexahedron polyhedra, hexagonal and tetragonal in outline, respectively. The administration *per os* of small amounts of cytoplasmic-polyhedrosis viruses and of inactivated polyhedrosis viruses partly prevents the occurrence of cytoplasmic polyhedrosis from the subsequently administered virus. The present paper is concerned with the interaction between the two viruses in a larva which was treated with cold or fed formalin with mulberry leaf, and also with the interaction between viruses fed to larvae and those injected into the hemocoele.

**Yokokawa S (1964). Further studies on the cytoplasmic polyhedrosis in silkworm larvae, *Bombyx mori* L., caused by inadequate treatments of eggs. *J. Sericult. Sci. Jpn*. 33:394-398. [Japanese]**

**Aizawa K and Fujiyoshi N (1964). The growth of *Bacillus thuringiensis* in dead larvae of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 33:399-402.**

The growth of *Bacillus thuringiensis* in dead silkworm larvae ($4^{th}$ to $5^{th}$ instar) has been investigated using a strain T84A. Well washed spore suspension was administered to silkworm larvae and larvae were killed by momentary dipping in boiling water. More obvious results than the case of a previous paper. (AIZAWA *et.al.*, JSSJ 31: 253 [1962]) regarding the growth of *Bacillus thuringiensis* and the formation of spores and crystal toxins were obtained particularly in larvae of the $1^{st}$ day (before feeding) of the $4^{th}$ instar. By injection of spores into larvae, the strain T84A grew in each larva and the formation of both spores and crystal toxins was recognized except larvae of the $3^{rd}$ day of the $5^{th}$ instar (subcutaneous inoculation). The strain T84A seems to grow and sporulate being accompanied with the formation of crystal toxins more easily than *Bacillus thuringiensis* var. *Thruringiensis* in dead silkworm larvae.

**Aizawa K and Furuta Y (1964). Resistance to polyhedroses in F1 hybrids between resistant and original strains in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 33:403-406.**

The selection of a silkworm strain resistant to virus induction has been continued by means of cold treatment. In the $17^{th}$ to $19^{th}$ generation, the survival rate of the selected strain was higher than that of the original (non-selected) strain and the incidence of polyhedroses of the former was lower than that of the latter. In F1 hybrids between selected and original strains, the survival rates

and the incidences of polyhedroses varied among silkworm rearing seasons and the forms of cross. These variations have no significance from genetical point of view.

**Aizawa K, Furuta Y and Choraku I (1964). Incidence of polyhedroses affected by feeding amounts of mulberry leaves after cold treatment in larvae of the silkworm, *Bombyx mori. J. Sericult. Sci. Jpn*. 33:417-418.**

It is already known that the incidence of polyhedroses in sw larvae after cold treatment decreases by starvation. However, the incidence of polyhedroses greatly increases by feeding a small amount of mulberry leaves which will cause a metabolic change in larvae to stimulate the induction of polyhedroses.

**Aruga H, Nagashima E and Takei R (1964). Generation to generation transmission of cytoplasmic-polyhedrosis virus and the role of chromosomal genes in *Bombyx mori* (LINNAEUS). *J. Sericult. Sci. Jpn*. 33:460-463.**

A few investigations on the generation to generation transmission of the cytoplasmic polyhedrosis virus and on the role of chromosomal genes of the host for the occurrence of the disease due to the transmitted virus have been carried out in the silkworm. It was observed that the cytoplasmic polyhedrosis larvae containing tetragonal polyhedra were induced by the cold treatment in the fourth or the fifth instars with a considerably high frequency in a few trials in which the second instar larvae had been inoculated with tetragonal cytoplasmic polyhedron viruses in the previous generation. On the other hand, no induction was observed in trials in which the tetragonal polyhedron viruses had not been inoculated in the previous generation. From such result it seems likely that the cytoplasmic polyhedrosis viruses of the silkworm are transmitted in an occult state from generation to generation through the egg.

The female moths of a few strains which had been inoculated *per os* with tetragonal polyhedra in the second instar were crossed with the male moths of the resistant or susceptible strains, respectively, and the $4^{th}$ or $5^{th}$ instar larvae of the next generation were exposed to cold (about 5°C) for 24 h. The incidence of cytoplasmic polyhedrosis with tetragonal polyhedra was higher in the progeny from the cross in which the resistant male moth was used. From such experimental results, it seems very likely that genes controlling the resistance play an important role for the occurrence of the cytoplasmic polyhedrosis in the fourth and fifth instars, which may be thought to be due to viruses transmitted through the egg.

**Asayama T (1964). A new strain of the nuclear polyhedrosis virus of the tussar silkworm, *Antheraea pernyi* (BOISDUVAL) (Lepidoptera, Saturnidae). *J. Sericult. Sci. Jpn*. 33:464-469.**

During the course of the rearing of the tussar silkworm, *A. pernyi*, a new type of nuclear polyhedra was found in Sep. 1962 at Aichi Sericultural Experiment Station. The new virus disease of tussar silkworm has a symptom closely similar to that of original nuclear polyhedrosis, but the polyhedron of the new virus strain was larger in size compared with the polyhedron of the original disease, and the size and shape of the polyhedron did not change by successive passages. The polyhedron revealed mainly triangular (tetrahedron) having round corners in outline and the mean size of the polyhedra from the last instar larva was 5.18μ, whereas 1-2μ in the original strain.

The polyhedron of the new strain was soluble in weak alkalies, but insoluble in weak acids, xylene, ether, chloroform, ethanol and water being alike to that of the original strain. It was stained with orange-G, picric acid, fuchsin, methylene blue, eosine, methyl green and safranin. The new virus strain was highly infectious for *Samia cynthia ricini* and *S. cynthia pryeir*, and the shape and size of the polyhedra formed in the larval bodies of these insect species were not

different from those found in *A. pernyi*. This virus was not infectious to *B. mori* as well as the original strain. In the double infection with the new and original virus strains, each type of polyhedra was formed in the cell nuclei of *A. pernyi*, but both kinds of polyhedra were not observed in the same nucleus.

**Ishikawa Y, Hayashida T and Ikawa A (1964). On the isolation of *Bacillus thuringiensis* from silkworm rearing houses in Aichi Prefecture. *J. Sericult. Sci. Jpn*. 33:480-483.**

In this paper the result on the isolation of *B.t* from the dust of silkworm rearing houses of farmers in Aichi Prefecture and the toxicity for the silkworm larvae were described. The isolation of *B.t.* was 33.3% out of 42 samples of the dust which were collected from rearing houses. Each isolate was toxic to silkworm larvae. The toxicity of *B.t.* was inactivated by submergence in boiling water for 5 min.

## Vol. 34 1965

**Ishikawa Y and Okino H (1964). Studies on the mode of the spread of viruses in the silkworm, *Bombyx mori* L. (I) On the infectivity of the cytoplasmic polyhedra passed through the intestinal canal of the domestic fowl, *Gallus gallus domesticus* BRISSEN. *J. Sericult. Sci. Jpn*. 34:21-23.**

Experiments which were conducted concerning the infectivity of the silkworm cytoplasmic polyhedra, passed through the intestinal canal of the domestic fowl (A Japanese bantam), showed that such polyhedra were still infectious to the silkworm.

**Hayashi Y and Kawase S (1965). Studies on the RNA in the cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. (I) Specific RNA extracted from cytoplasmic polyhedra. *J. Sericult. Sci. Jpn*. 34:83-89.**

The nucleic acid extracted from the cytoplasmic polyhedra of the silkworm with phenol method revealed fibrous masses besides powdery precipitates in alcohol. The fibrous nucleic acid was further fractionated with methylated albumin column. From the elution pattern, it has been known that the nucleic acid is mainly consisted of the fraction being eluted at 0.60-0.65 M NaCl concentration. The fractionated nucleic acid was positive to orcinol reaction, but negative to diphenylamine and indole reactions, and furthermore sensitive to RNase but resistant to DNase. From these results, it will be concluded that the nucleic acid is a specific RNA occurred in the virus particles in the polyhedra. The specific RNA is extractable from both icosahedron and hexahedron inclusions of cytoplasmic polyhedrosis. They were denominated tentatively as IPB and HPB-RNA, the prefixes IPB and HPB being derived from icosahedral and hexahedral polyhedra of *Bombyx mori*. It appears that the powdery precipitates from the polyhedra is mainly consisted of oligo nucleotides as revealed in the elution pattern.

**Hayashi Y and Kawase S (1965). Studies on the RNA in the cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. (II) Base composition of the specific RNA extracted from cytoplasmic polyhedra. *J. Sericult. Sci. Jpn*. 34:90-94.**

The base compositions of the IPB- and HPB-RNA were revealed as AU type, but the nucleotide fraction was abundant in purine bases. Furthermore, the IPB- and HPB-RNA each showed base pairing: A/U and G/C revealed about 1. From above mentioned results, it seems highly probable that the IPB- and HPB-RNA are additional double stranded RNA of large molecular weight besides the RNA in wound tumour virus and reo virus.

**Ishihara R, Fujiwara T and Sawada N (1965). Regression of the numbers of infected moths and died larvae in the pebrine infection of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 34:121-124. [Japanese]**

**Tanaka S and Shimizu T (1965). Temperature effects on the multiplication of the flacherie virus in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 34:125-127. [Japanese]**

**Ishihara R and Hirose Y (1965). A preparation method for the suspension of muscardine spore. *J. Sericult. Sci. Jpn*. 34:129-130. [Japanese]**

**Hayashi Y, Hayashi Y and Kawase S (1965). Studies on the RNA in the cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. (III) Comparison between IPB- and HPB-RNA. *J. Sericult. Sci. Jpn*. 34:167-170.**

Identification of IPB- and HPB-RNA was carried out by methylated albumin column chromatography and with the pattern of ultracentrifugation. No significant difference was observed between the elution pattern of the column chromatography of IPB- and HPB-RNA. As for ultracentrifugation pattern, both the IPB- and HPB-RNA showed single peak of 17 S in a high concentration and two peaks of 14 S and 17 S in a low concentration. From these results, it is highly probable that IPB and HPB-RNA are closely related each other.

**Hayashi Y and Kawase S (1965). Studies on the RNA in the cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. (IV) Subsellular distribution. *J. Sericult. Sci. Jpn*. 34:171-175.**

This paper deals with the subcellular distribution of RNA of the midgut of the silkworm infected with cytoplasmic polyhedrosis virus. The results obtained are as follows:

When silkworm was infected with cytoplasmic polyhedrosis virus, the ratio of RNA content in mitochondrial fraction of diseased midgut decreased markedly, in the soluble fraction, on the other hand, increased strikingly, without any detectable difference on both nuclear and microsomal fraction. RNA of each fraction was analysed chromatographically with methylated albumin column. In the nuclear, mitochondrial and microsomal fractions of diseased midgut, IPB-RNA occupied 16.0, 8.1 and 5.3% to the total RNA respectively. It is of interest that a component like IPB-RNA was observed in microsomal fraction of healthy midgut amounting to 7% of the total RNA.

**Hayashi Y and Kawase S (1965). Studies on the RNA in the cytoplasmic polyhedra of the silkworm, *Bombyx mori* L. (V) Changes in fractionated RNA of the midgut. *J. Sericult. Sci. Jpn*. 34:244-250.**

The present investigation was undertaken to see the changes in the fractionated RNA during the progress of cytoplasmic polyhedrosis. In the healthy midgut, nucleotide content was inclined to decrease during the larval growth, while s-RNA content varied according to larval growth, lower at the beginning, higher after the middle of the instar. r-RNA occupied about 80% of total RNA throughout the larval growth. In the case of the diseased midgut, the nucleotide tended to decrease until 48 h after infection, thereafter increased markedly and reached about 21%. s-RNA content followed roughly the same curve as that of the healthy one, but decreased at 144 h after infection, while r-RNA decreased gradually and reached to 50-60% of total RNA. On the contrary, IPB-RNA increased gradually and reached about 12% at 144 h after the infection. From the results of the changes in IPB-RNA, it will be supposed that virus multiplicates gradually in the beginning of the infection (until 48 h after infection), then increased abruptly in the middle stage from 48 – 96 h, thereafter the multiplicative rate was lowered gradually.

Base composition of the fractionated RNA revealed that s-RNA in the healthy midgut showed high guanine and low uracil in content. In the diseased midgut, the nucleotide showed low purines and high pyrimidines in content. Remarkable difference between the healthy and diseased midgut was observed on the uracil content of s- and r-RNA. The base composition of IPB-RNA from the diseased midgut showed AU type as well as the IPB-RNA extracted from cytoplasmic polyhedra. The base composition of the RNA in the diseased midgut changes from GC to AU type during the progress of the disease. This phenomenon seems to indicate the increase of IPB-RNA in the midgut during the progress of cytoplasmic polyhedrosis.

**Watanabe H and Nagano H (1965). Resistance to *per oral* infection with polyhedrosis virus in the normal, *od*-translucent and lemon-yellow larvae of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 34:251-256.**

Biochemical mutants called lemon yellow (*lem*), which contains xanthoprotein-B in the integument cuased by the abnormal metabolism of pteridine, and *od*-translucent (*od*), of which *od* oily gene is physiologically pleitropic and expresses its action in almost all of the organs and tissues of ectodermal origin, were tested in the experiment on the susceptibility to *per oral* infection with nuclear and cytoplasmic polyhedrosis viruses. Since the experiment was done to clarify whether these mutant genes have any effect on the virus infection, several strains which segregate the normal, lemon-yellow and *od*-translucent larvae with isogenic backgrounds had been established before he experiment. The experimental results indicated that both lemon-yellow and *od*-translucent larvae showed no clear difference on the susceptibility compared with the respective normal segregants to *per oral* infection with viruses of nuclear and cytoplasmic polyhedroses. On the other hand, as it was revealed that the susceptibility was much more affected by the difference of genetic background than by the action of *lem* gene or *od* gene, these mutant genes seemed to play a minor role, if any, on the *per oral* infection with polyhedrosis virus.

**Iwashita Y (1965). Histo- and cyto-pathological studies on the midgut epithelium of the silkworm larvae infected with infectious flacherie. *J. Sericult. Sci. Jpn*. 34:263-273.**

Histo and cyto- pathological studies were made on the midgut epithelium of the silkworm larvae infected with the flacherie virus. The results may be summarized as follows:

In the infected larvae the histological changes occur at first in the goblet cells and then in the cylindrical cells. In the early stage of infection, the nucleus of goblet cells is strongly stained with methyl green and shows Feulgen positive, and the cytoplasm is stained with pyronine. In the late stage of infection the cytoplasm is thickened and thereafter the pyronine stainability decreases. At this stage the number of mitochondria decreases. When the disease is far advanced, the infected goblet cells are liberated into the midgut lumen.

The nuclei of infected cylindrical cells are well stained with methyl green and shows Feulgen positive, the cytoplasm are deeply stained with pyronine at first. However, the stainability reduce at later stage. And in the immediate perinuclear region the basophilic body (containing RNA) which is stained with pyronine has been produced. The production of basophilic body are observed in the cylindrical cells, anterior or middle, of the midgut. The nuclei, on the other hand, shows, the characteristic hyperchromatosis; that is the chromatin material is distributed equally in the enlarged nucleus. In the later stage of infection, cells show the varieties of cytoplasmic and nuclear damages. The cytoplasm become vacuolated and the mitochondria decrease in number. The hypertrophied nuclear chromatin material may become the gathered masses, forming the irregular accumulations. Eventually, the infected cells degenerate and liberate into the midgut lumen, adhering to peritrophic membrane together with degenerated and liberated goblet cells and forming membraneous material. Consequently, it is observed that peritrophic membrane has become remarkably thickened.

Alkaline phosphate activity of the cylindrical cells is rather weak as compared to that of healthy silkworm. However, only the hypertrophied nuclei show strongly positive reaction. It is presumed that the virus infects goblet cells at first and then cylindrical cells. Moreover, cells in the anterior portion of the midgut are infected at first and then the infection gradually spread to cells of the middle and posterior portions. Double infection with viruses of nuclear polyhedrosis and infectious flacherie is observed in larvae. Many larvae mixedly infected with the viruses of cytoplasmic polyhedrosis and infectious flacherie are observed. The polyhedra formed in the cylindrical cells are very small in size and few in number as compared with the larvae infected alone with cytoplasmic polyhedrosis virus.

**Nakasone S (1965). Acrylamide gel electrophoresis of alkaline phosphatase in the midgut of the cytoplasmic-polyhedrosis silkworm (*Bombyx mori*). *J. Sericult. Sci. Jpn*. 34:281-283.**

The author has performed acrylamide gel electrophoresis of alkaline phosphatase in the midgut of the larva infected with the cytoplasmic polyhedrosis virus of the silkworm. The results obtained are as follows:

Five bands (A, B, C, D and E) were detected in zymogram with the enzyme solution from the healthy larvae, even though E was seen as very light band, while B and C bands were detected as trace amount and band D became weaker with the enzyme solution from infected larvae.

**Ishisaka Y and Asayama T (1965). Studies on the relation between polyhedrosis of the wild insects and the silkworm, *Bombyx mori* (LINNAEUS). (VI) Cross-transmission of the nuclear-polyhedrosis virus among seven species of lepidopterous insects. *J. Sericult. Sci. Jpn*. 34:343-350.**

Studies were carried out on the cross transmission of the nuclear-polyhedrosis viruses among *Bombyx mori*, *Theophila mandarina*, *Samia cynthia pryeri*, *Samia cynthia ricini*, *Antherae pernyi*, *Antheraea yamamai*, and *Dictyoploca japonica*. The results obtained were as follows:

The nuclear polyhedrosis virus of *T. mandarina* was transmissible to *S. cynthia pryeri* and *S. cynthia ricini*, but not to *D. japonica*; the virus of *S. cynthia pryeri* to *A. pernyi*, *A. yamamai* and *D. japonica*; that of *A. pernyi* to *S.* cynthia *ricini* and *D. japonica*, but not to *t. mandarina;* that of *A. yamamai* to *S. cynthia ricini*, but not to *t. mandarina*; that of *D. japonica* to *S. cynthia ricini*, *A. pernyi*, and *A. yamamai*, but not to *T. mandarina;* that of *D. japonica* to *S. cynthia ricini*, *A. pernyi*, and A. yamamai, but not to *T. mandarina*.

**Aruga H and Hashimoto Y (1965). Inference between the UV-inactivated cytoplasmic-polyhedrosis viruses in the silkworm, *Bombyx mori* (LINNAEUS). *J. Sericult. Sci. Jpn.* 34:351-354.**

To determine the effect of inactivated virus by UV-irradiation on the multiplication of active virus, the incidence of cytoplasmic polyhedrosis was examined in the case of the administration of both inactivated and activated viruses.

It was observed that the incidence of cytoplasmic polyhedrosis was reduced when inactivated icosahedron virus was administered 3 times 12 h before the administration of active hexahedron viruses, whereas such clear cut reduction of the incidence of cytoplasmic polyhedrosis by challenge virus was not observed when inactivated virus was administered once.

The experimental results show that in the case of cytoplasmic polyhedrosis of *B. mori* the administration of inactivated polyhedrosis viruses by UV-irradiation can prevent the occurrence of cytoplasmic polyhedrosis from subsequently administered virus.

**Yamazaki H, Yamada T and Kobayashi A (1965). A silent infection of the infectious flacherie (F) of silkworms. *J. Sericult. Sci. Jpn*. 34:355-358.**

The feces deposited from each of ten silkworms which had been reared individually and grew up the healthy adult were ground down filtered. The 1/2 x $10^{-1}$ fluid of this filtrate was smeared on mulberry leaves, which were given to 100 newly hatched larvae. As the result, in two of ten larval groups feeding on the smeared leaves, the majority of the larvae were suffered from the flacherie disease (F). Further the feces from $5^{th}$ or $6^{th}$ day old $5^{th}$ instar larvae which were in very good health, being bred by 18 excellent Sericulturists, were given to the newly hatched larvae by the same method as above. Then feces from these larvae reared by the 10 farmers showed to cause the flacherie disease in the $4^{th}$ instar of the tested larvae. This fact suggests that there is such a phenomenon that the silkworms are not affected with the flacherie holding a lot of the germs in their bodies. We call it “silent infection”.

**Miyajima S and Kawase S (1965). The inhibitory effect of 5-flourouracil on the outbreak of infectious flacherie in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn*. 34:359-365.**

The effect of antiviral chemicals and their related substances upon the infectious flacherie (viral flacherie), cytoplasmic polyhedrosis and polyhedroses caused by treatment with low temperature was investigated. 21 kinds of chemicals, *ie*, 8-azaadenine, 8-azaguanine, benzimidazole, 2-thiouracil, thymine, uracil, mitomycin, kinamycin, tetracycline, antimycin-A, chloramphenicol, bellilium sulphate, strontium sulphate, 8-hydroxyquinoline, γ-globulin, SDS, acridine orange and diethyl-2-thiocarbamate were used for these experiments. It has been found that the progression of the infectious flacherie caused by the virus inoculation was inhibited significantly when the larvae were fed with mulberry leaves sprayed with aqueous suspension of 5-fluorouracil (5 x $10^{-2}$ percent or 1 x $10^{-2}$ percent) prior to and after the virus inoculation. Moreover, when uracil solution was mixed into 5-flourouracil solution in the same volume, the inhibiting effect was disappeared.

**Komori S (1965). Studies on the refractive index of the body fluid of the silkworm larvae infected with the infectious flacherie or the cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn*. 34:366-368.**

All except 5-fluorouracil showed no significant inhibiting effect for the infectious flacherie. Chemicals tested in the present experiment showed no inhibiting effects on cytoplasmic polyhedrosis caused by the virus inoculation or polyhedroses caused by treatment with low temperature.

**Tanaka S (1965). On the nuclear polyhedrosis occurred during aseptic raising of tussar silkworm, *Antheraea pernyi*, GUERIN-MNEVILLE (Preliminary note). *J. Sericult. Sci. Jpn*. 34:369-370. [Japanese]**

**Ishikawa Y and Okino H (1965). Studies on the toxicity of *Bacillus thuringiensis*, passed through the intestinal canal of the domestic fowl, to silkworm larvae. *J. Sericult. Sci. Jpn*. 34:371-373.**

*Bacillus thuringiensis* passed through the intestinal canal of the domestic fowl (a Japanese bantam *Gallus gallus domesticus* BRISSEN) had toxicity to silkworm larvae. It was recognized that *B. t.* grows on the agar culture, prepared with the excreta and the soil of fowl house and has a considerable toxicity to silkworm larvae.

**Kawakita T, Ishisaka T, Watanabe T and Hayashi K (1965). Bacteriological studies on the bacterial groups isolated by the anaerobic culture method from silkworm larvae infected with flacherie and unidentified bacteria taken on mulberry leaves. *J. Sericult. Sci. Jpn*. 34:375-384. [Japanese]**

**Aruga H, Tanaka S and Shimizu T (1965). Interference by the infectious flacherie virus on the cytoplasmic-polyhedrosis virus in the silkworm, *Bombxyx mori* (LINNAEUS). *J. Sericult. Sci. Jpn*. 34:385-390.**

Interference between the infectious flacherie virus and the cytoplasmic polyhedrosis virus in *Bombyx mori* L. was investigated using the second, third and fourth instar larvae. The results obtained are as follows:

The administration *per os* of the infectious flacherie virus can prevent the occurrence of the cytoplasmic polyhedrosis from subsequently administered cytoplasmic polyhedrosis virus. No interference was observed when the infectious flacherie virus was administered after the administration of the cytoplasmic polyhedrosis virus. The interference by the infectious flacherie virus on the cytoplasmic polyhedrosis virus was clearly observable when large dose of the infectious flacherie virus was administered. When the dose of the cytoplasmic polyhedrosis virus was relatively small, the interference phenomenon was more clearly observable in the twice inoculation of $10^{-4}$ dose than in the once inoculation of $10^{-2}$ dose of the infectious flacherie virus.

**Aruga H and Watanabe H (1965). The effect of high temperature on the infection with cytoplasmic polyhedrosis viruses and on interference between viruses in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 34:391-394.**

Studies were carried out to know the effect of high temp on the infection with cytoplasmic polyhedrosis viruses and on the interference between tetragonal polyhedron virus and hexagonal polyhedron virus in the silkworm. The incidence of cytoplasmic polyhedrosis was higher in high rearing temp (36°C, 24 h) than in low temp (25°C, 24 h) before or after the administration of the tetragonal polyhedron virus and or hexagonal polyhedron viruses. In the case of the rearing at 25°C for 24 h the administration *per os* of the tetragonal polyhedron viruses prevented the occurrence of cytoplasmic polyhedrosis from subsequently administered hexagonal polyhedron viruses. The phenomenon is though to be due to the interference of the tetragonal polyhedron virus with the infection by the hexagonal polyhedron virus. Whereas in the case of rearing at 36°C for 24 h no such prevention was observed, but rather the occurrence of the cytoplasmic polyhedrosis by the tetragonal polyhedron viruses previously administered was remarkably prevented.

## Vol. 35 1966

**Ishikawa Y and Okino H (1966). The infectivity of Silkworm Nuclear Polyhedra Passed through the Intestinal Canal of the Domestic Fowl, *Gallus gallus domesticus* B. *J. Sericult. Sci. Jpn*. 35:21-22. [English]**

Ishikawa and Okiono reported that the cytoplasmic polyhedra of the silkworm *Bombyx mori* L., which had passed throught the intestinal canal of the domestic fowl retained infectivity for silkworm larvae. These facts are important not only from the viewpoint of the spread of the polyhedra but also from the standpoint of prevention of virus dieases in the sericulture. The present study deals with the infectivity of the silkworm nuclear polyhedra swallowed by domestic fowl.

**Uzigawa K and Aruga H (1966). On the selection of resistant strains to the infectious flacherie virus in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 35:23-26. [Japanese]**

**Watanabe H (1966). Genetic resistance to peroral infection with cytoplasmic polyhedrosis virus in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 35:27-31.**

One of the most resistant silkworm strains to *per oral* infection with the cytoplasmic polyhedrosis virus is Daizo, while the Okusa strain is hightly susceptible. The mode of inheritance of resistance in the Daizo strain has been investigated in the present study. The resistance to virus infection was tested with individuals of the F1, F2 and backcrossed hybrids obtained from crosses between the two inbred strains Daizo, and Okusa.

The results of the test revealed that the resistance of F1 hybrids of Daizo x Okusa were nearly the same as that of Daizo, while the resistance of F2 hybrids and the backcrossed hybrids to Okusa stood approximately intermediate between the two inbred strains. This indicated that the resistance in the Daizo strain was inherited as a complete dominance. Although it is possible that the resistance to *per oral* infection with the cytoplasmic polyhedrosis virus in the silkworm is of polygenic type, the results suggest that a highly resistant strain like Daizo may have a dominant major gene controlling the resistance. No marked difference in the resistance to polyhedrosis infection was observed in the reciprocal hybrids obtained from the F1 and back crosses. There was also no indication of a maternal effect on the inheritance of resistance.

**Ayuzawa C and Furuta Y (1966). Susceptibility of the silkworm (*Bombyx mori* L.) to the nuclear polyhedrosis virus and the role of the inhibiting activity of the gut-juice. *J. Sericult. Sci. Jpn*. 35:66-70.**

The present paper deals with the susceptibility of larvae of the silkworm to the nuclear polyhedrosis (N) virus in relation to the inactivation activity of the gut juice, since it had been suggested that such an activity found in the gut juice was one of the factors concerning the occurrence of nuclear polyhedrosis by the low temp treatment 5°C 24 h.

When the larvae were exposed to the low temp (5°C 24 h), just after the 4$^{th}$ ecdysis, their susceptibility to per os infection with N-virus was highly increased, as compared to the 3$^{rd}$ ecdysis and those treated one day after the 3$^{rd}$ ecdysis. The pH value of the gut-juice of larvae exposed to low temp was always lower than that of control larvae, and the inhibiting activity of the gut juice of the treated larvae was much lower than that of larvae which had not been exposed to the low temperature. When the newly moulted 5$^{th}$ instar larvae had been forced to vomit the gut juice by applying an electric shock, their susceptibility to N-virus was markedly increased.

From these results it was considered that the occurrence of nuclear polyhedrosis after the low temp treatment seemed to be, at least partly, due to the decrease in the inhibiting activity of the gut-juice, of the larvae thereafter might be infected with the contaminating pathogen under rearing circumstances.

**Hosada S, Azuma T and Yamazaki H (1966). Relation between occurrence of the flacherie disease and inoculation time and concentration of its pathogen to the silkworm. *J. Sericult. Sci. Jpn*. 35:71-77. [Japanese]**

**Hosada S, Azuma T and Yamazaki H (1966). Infection of the flacherie disease in the silkworm rearing seat. *J. Sericult. Sci. Jpn*. 35:78-80. [Japanese]**

**Okubo N (1966). Inapparent infection with the infectious flacherie virus in the moth of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 35:81-84.**

The author observed the pathogenicity in the silkworm moth, normally emerged and oviposited, which had been fed with the flacherie virus just after the 4$^{th}$ molting, but recognized

no pathogenicity in the control, which had not been fed the virus. The virulence of moth was in roughly proportion to the dosage of the virus suspension being administered in the 5th instar.

**Aoki J (1966). Pathogenic fungi and insect pests on hibernating pupae of the fall webworm, *Hyphantria cunea* DRURY in mulberry fields. *J. Sericult. Sci. Jpn*. 35:141-144. [Japanese]**

**Yamazaki H, Yamada T and Kobayashi A (1966). Some observations on the inactivation of the pathogen of flacherie (F) by means of manuring with slaked lime. *J. Sericult. Sci. Jpn*. 35:158-160.**

Testing by means of bioassay method and using just hatched normal silkworm larva, it was found that flacherie (F), when immersed in 5% solution of slaked like for thirty minutes, was perfectly inactivated. Moreover, it was also proved that the virus was inactivated within a day when it was mixed with the soil of mulberry field manured with the milk lime. Hence it may be suggested either the scattering of slaked lime upon the silkworm rearing bed, or the manuring of the mulberry field with the lime, is an effective method for the purpose of inactivation of flacherie virus (F).

**Yamazaki H, Yamada T and Kobayashi A (1966). On the infection of flacherie (F) through the scales of silkworm moth and the dust of the room of silkworm egg-laying. *J. Sericult. Sci. Jpn*. 35:161-164.**

In the present paper, it was observed that the scales of the silkworm moth collected from the bad Sericultural crop and the dusts collected from the room of the silkworm egg laying, the same collected from inside the refrigerator for male moths, and from the silkworm egg drying room for artificial hatching, often have a possibility of infection by flacherie (F) to the silkworm larva when reared and testified by means of bioassay method.

**Ishikawa Y, Tanabe H, Nakayama C and Asayama T (1966). Studies on the nuclear polyhedrosis of *Porthesia xanthocampa* D. *J. Sericult. Sci. Jpn*. 35:174-180.**

In June of 1964, the nuclear polyhedrosis of *p. x.* was found in older larvae at the mulberry field in Aichi-ken Sericultural experiment station. The polyhedra were observed tetragonal or triangular in outline. The sizes of the polyhedra were 1 to 3 μ and the average diameter was 1.79μ. The polyhedra were stained with picric acid, Ziel's fuchsin, methylen blue, etc and were soluble in weak alkaline, but insoluble in ethanol, ether and water. This virus has a considerable pathogenicity for the larvae of *P.x.* and the incubation period from infection to death was about 10 days at the rearing temp of about 24 to 25°C. The nuclear polyhedrosis virus of *P.x.* was not transmissible to *Bombyx mori*, *Dictyoploca japonica*, *Papilio xuthus* and *Canephora asiatica.* The nuclear polyhedrosis virus of *B.m.*, Hyphantria *cunea*, *Antheraea pernyi*, *Dictyoploca japonica* and the cytoplasmic polyhedrosis virus of *B.m.* were not transmissible to *P. xanthocampa.*

**Ishikawa Y and Tanabe H (1966). Cytoplasmic Polyhedrosis of *Porthesia xanthocampa*. *J. Sericult. Sci. Jpn*. 35:181-185. [English]**

Cytoplasmic polyhedrosis of *Porthesia xanthocampa* was found in old larvae in the mulberry field at Aichi-ken sericultural experimental station. It was observed that there is wide variation in the size of polyhedra. The average was about 1.5 microns, but many were less than 1 micron and a few were more than 4 microns. The shapes of larger polyhedra were seen as hexagonal and tetragonal having blunt corners, but the shape of smaller ones could not be definitely determined but were looking spherical in outline. It was shown that the cytoplasmic polyhedrosis virus has a considerable pathogenicity for the larvae of *Porthesia xanthocampa* and

the incubation period from infection to death was 7 to 14 days during summer. The cytoplasmic polyhedrosis virus of *Porthesia xanthocampa* has no infectivity for silkworm larvae.

**Hosada S, Azuma T, Matsuzawa N and Yamazaki H (1966). Pathogenic activity of dungs excreted from domestic animals which ate flacherie germs of the silkworm. *J. Sericult. Sci. Jpn*. 35:186-188. [Japanese]**

**Kawakita T, Watanabe T, Ishisaka T and Hayashi K (1966). On the susceptibility to antibacterial agents in various bacteria isolated from the silkworm larvae infected with flacherie and unidentified bacteria taken from mulberry leaves. *J. Sericult. Sci. Jpn*. 35:248-252. [Japanese]**

**Miyajima S and Kawase S (1966). The inhibitory effect of 5-flourouracil on the outbreak of infectious flacherie in the silkworm, *Bombyx mori*. (II) The effective concentration of 5-flourouracil and the effect of thymine on the 5-flourouracil function. *J. Sericult. Sci. Jpn*. 35:253-256.**

Effective concentrations of 5-fluorouracil (FU) to inhibit the outbreak of infectious flacherie were from the concentrations of $5 \times 10^{-4}$ to $5 \times 10^{-5}$ M, when administered prior to and after the virus infection. When an effective dosage of FU was used together with the same molar concentration of thymine as FU, the function of the chemical disappeared.

**Miyajima S and Kawase S (1966). The inhibitory effect of 5-flourouracil on the outbreak of infectious flacherie in the silkworm, *Bombyx mori*. (III) On some administration methods of 5-flouracil. *J. Sericult. Sci. Jpn*. 35:257-261.**

5-fluorouracil (FU) functioned to inhibit the incidence of infectious flacherie when it was administered simultaneously or shortly after the virus inoculation, but the administration before inoculation resulted in fuitlessness. The problems as to when FU should be used after the virus inoculation was disobeyed. The results revealed that the shorter the time after inoculation the less the incidence of infectious flacherie, and furthermore, the more the number of times administered, the less the incidence of infectious flacherie.

**Asayama T (1966). Cytoplasmic polyhedrosis of the mulberry silkworm, *Theophila mandarina* (MOORE) (Lepidoptera, Bombycidae). *J. Sericult. Sci. Jpn*. 35:267-272.**

Mulberry silkworms, *Theophila mandarina*, affected with CPV were found in a group of larvae collected from mulberry field of Aichi Ken Sericultural Experimental Station. The larvae affected with the cytoplasmic polyhedrosis virus exhibited external signs and symptoms, *ie*, loss of appetite, faint movement, indication of diarrhea, nevertheless no change was seen in colour of the larval body.

On dissecting the diseased larva, the alimentary canal, especially the midgut was opaque white in appearance. On the histological observations, the cytoplasm of epithelial cells contained variable size of polyhedra ranging from 0.4 to 8.6μ. The shapes of polyhedra were mainly tetragonal in outline, but a few were hexagonal or irregular in shape. The shape and size of polyhedra formed in one cell were nearly uniform. The polyhedra were also observed in the regenerative cells of the midgut. *Bombyx mori* was susceptible to the CPV of *T. mandarina* and the both types of CPV of *B.m., ie*, hexagonal and tetragonal in outline of their polyhedra were infectious for *T. mandarina* respectively.

**Watanabe H (1966). Probit analysis for determining the resistance of the silkworm, *Bomyx mori* L. to the infection by cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn*. 35:289-295. [Japanese]**

**Utsumi S, Kurisu K and Ichikawa Y (1966). Actions of β-propiolactone on the pathogenic bacteria and fungi of the silkworm, *Bomyx mori* L. *J. Sericult. Sci. Jpn*. 35:393-397.**

This work was carried out to investigate the disinfecting effects of β-propiolactone (PL) on the pathogenic bacteria and fungi in the silkworm diseases. The spore forming bacteria, *Bacillus thuringiensis* var. *sotto*, were killed by immerse in the aqueous solution of PL of 1.3 x $10^{-1}$ M at 25°C for 20 min. Immersion in the PL of 3.3 x $10^{-2}$ M at 25°C for 10 min. was sufficient to kill the non-spore forming bacteria, *Serratia marcescens* and *Pseudomonas aeruginosa*. The hydrolyzed substance of PL, β-hydroxy priopionic acid (HPA), had disinfecting power on those bacteria at the concentration of 7.5 x $10^{-1}$ M. Among the pathogenic fungi studied, *Ospora destructor*, *Isaria farinosa*, *Aspergillus oryzae* and *A. niger*, *O. destructor* was the most resistant to PL. *O. destructor* was killed, however, by the immerse in 1.3 x $10^{-1}$M PL for 5 min. or 3.3 x $10^{-2}$ M for 30 min. *Bacillus thuringiensis* var. *sotto* was perfectly inhibited to grow and killed with 3.0 x $10^{-2}$M PL in Bouillon medium (pH 7.2) and partially inhibited to grow with lower concentration than 3.0 x $10^{2}$M.

**Utsumi S, Kurisu K and Ichikawa Y (1966). Actions of β-propiolactone on the pathogenic viruses of the silkworm, *Bomyx mori* L. *J. Sericult. Sci. Jpn*. 35:398-404.**

This paper has dealt with the inactivation of nuclear polyhedra, nuclear polyhedrosis virus and infectious flacherie virus of silkworm, *Bombyx mori* L., by β-propiolactone (PL) and the effects of administration of inactivated virus or nuclear polyhedra to the silkworm as to the susceptibility to the virus diseases.

Nuclear polyhedra: Purified and dried polyhedra were employed. Nuclear polyhedra were inactivated with 1.5 x $10^{-1}$M of PL and 21°C for one hour. Nuclear polyhedrosis virus: Virus was prepared from nuclear polyhedrosis blood by weak alkaline treatment. The virus was inactivated with 1.2 x $10^{-1}$M of PL at 21°C for 1 h. Infectious flacherie virus: The supernatant which was prepared by homogenizing and centrifuging the infected larvae was employed as a virus sample. The virus was inactivated with 1.2 x $10^{-1}$M at 28°C for one hour. The decreasing effects of susceptibility to the active virus infection was observed in the silkworm which was preliminarily administrated the inactivated nuclear polyhedra or infectious flacherie virus.

**Watanabe H (1966). Some aspects on the mechanism of resistance to peroral infection by cytoplasmic-polyhedrosis virus in the silkworm, *Bomyx mori* L. *J. Sericult. Sci. Jpn*. 35:411-417.**

Interstrain difference in dosage-infection response of silkworm to the CPV was determined and analysed to gain some information about the mechanism of host resistance. Comparison of dosage infection responses of larvae following the inoculation of the virus particles by ingestion and by subcutaneous injection revealed that much of the virus ingested did not invade into the susceptible epithelium cells of the midgut. Because considerably less virus was required to produce the same amount of infection when larvae were injected with virus particles into the body cavity. And no infectious virus was generally detected in the mid guts of those larvae, which had been alive without suffering from the virus disease. No significant correlation was observed between the interstrain difference in susceptibilities to *per oral* and sub-dermal infections.

All these results obtained support the interpretation that the host resistance to *per oral* infection of the virus depends much on the inhibitory mechanism in the gut lumen against invasion of the virus into the midgut cells, and less on the suppression of virus multiplication within the cells.

**Watanabe T, Tsutsui R and Iwahana H (1966). Toxic crystals produced by *Bacillus thruringiensis* var. *sotto* (I) Separation and properties of crude toxin *J. Sericult. Sci. Jpn*. 35:418-422.**

Crystalline inclusions which contain toxic substances for lepidopterous larvae occur in sporulating cells of *Bacillus thuringiensis* var. *sotto* ISHIWATA and have been obtained in a relatively pure state by BATESON'S method from sporulated culture. The crystalline inclusions were soluble in alkaline solution above pH 0.5 and lost the toxicity by heating at 100°C for 30 min, or treatment with the usual protein denaturants, *ie*, $HgCl_2$, TCA, formalin and 0.5 N NaOH.

Crude toxin was separated from the crystalline inclusions by extraction at pH 11.5 with alkaline solution, followed by precipitation at pH 4.4 with acetate buffer. The crude toxin was inactivated by heating at 65°c for 60 min, or treatment with methanol, ethanol, acetone besides the usual protein denaturants. LD50 of the crude toxin was 1.2µg/g for silkworm larvae.

**Watanabe T, Tsutsui R and Iwahana H (1966). Toxic crystals produced by *Bacillus thruringiensis* var. *sotto* (II) Sedimentation, diffusion, electrophoresis and fractionation by column chromatography of crude toxin. *J. Sericult. Sci. Jpn*. 35:423-426.**

In order to investigate the homogeneity of the crude toxin extracted from crystalline inclusions of *Bacillus thuringiensis* var. *sotto*, the present investigation was carried out by means of sedimentation, diffusion, electrophoresis and fractionation with Sephadex G-200 and DEAE-cellulose. The crude toxin contained at least two major protein components, however, it was found that the toxic substance in the crude toxin was minor component other than the major components.

**Eguchi M, Masayama T and Nishimura M (1966). Changes in electrophoretic pattern of proteins in several tissues of the silkworm, *Bombyx mori* L., during metamorphosis. *J. Sericult. Sci. Jpn*. 35:435-443.**

By means of improved disc-electrophoresis (discontinuous) the separation of proteins extracted from the blood, intestine, fat body, integument and ovary of the silkworm was carried out. Rapidly moving bands of the extract from the intestine became very faint at the pre-pupal stage and the staining intensity of these bands rose again at the early to middle pupal stage. The marked changes in the electrophoretic pattern was observed in the integument, that is, fast migrating three bands which could be detected till the prepupal stage were not discernible at the 4 days after pupation. Whereas, rather intense protein staining was found as to slow moving bands at the pupal stage.

The fat body contained very light protein bands at the early to middle parts of the $5^{th}$ instar. Several dense and weak bands appeared at the prepupal stage, and there was no difference in electrophoretic patterns during development. The ovary had several bands, one of which was represented very faintly at the middle pupal stage and intensely at the late pupal stage. Blood proteins occur in at least eight components, and the bands were deeper in the female than in the male at the same period. By the application of the thin layer polyacrylamide gel electrophoresis, it was shown that the fast migrating band b became light in colour after mature larval period, band d was most intense at the prepupal stage and band e which could not be detected in the male blood at the prepupal stage was found in the female at the same period. By the lipid staining of gels after electrophoresis of extracts from the ovary or blood, two bands which are considered to be lipoprotein were observed respectively.

**Kitano M, Onishi M, Yamaguchi C, Utsumi S, Kurisu K and Ichikawa Y (1967). Effect of β-propiolactone spraying on the rearing of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 35:445-448.**

Giving an answer to the question whether or not β-propiolactone (PL) is one of disinfecting agent in the silkworm rearing, we have reared the 5th instar larvae sprayed with the chemical of 2.5, 1.25 or 0 per cent concentration on their rearing seats once a day. Pupating rate in the worms sprayed with 2.5 per cent PL increased about 10 per cent comparing with those in 1.25 or 0 per cent lots. The cocoon quality of those worms had not conspicuous effects by PL spraying. From the results mentioned above, it may be suggested that the larvae are prevented from some infectious diseases by the treatment of 2.5 per cent PL. This problem should be discussed at a much wider scale in the future, based on the results obtained from a variety of experiments.

## Vol. 36 1967

**Tanaka S, Shimizu T and Aruga H (1967). Midgut nuclear polyhedrosis forming polyhedra in the nucleus of midgut cell of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 36:1-9.**

A new type of polyhedrosis was found in which hexagonal polyhedra were contained within the nucleus of the cylindrical cells of midgut in the silkworm. The external symptom of diseased midgut of this polyhedrosis does not show the whitish colour symptom different from the cytoplasmic polyhedrosis. In the case of both the *per os* infection and injection, no polyhedron was observed in the cell nuclei of other tissues of the diseased silkworm. When polyhedra formed in the nuclei of midgut cells were measured under a light microscope, they ranged in diameter size from 20.7 to 3.5 microns. The number of polyhedra contained in one nucleus was from one to four. The polyhedra can be stained with bromophenol blue, orange G and eosin in the case of pretreatment with 1N HCl at 60°C and also with pyronin when vitally stained with pyroninmethyl green.

**Watanabe T, Iwahana H, Ogi H and Tsutasui R (1967). Toxic crystals produced by *Bacillus thuringiensis* T84A1 (I) Separation and properties of crude protein. *J. Sericult. Sci. Jpn*. 36:11-15.**

A mixture of sucrose, triflurotrichloroethane and cyclohexane was used as a solvent for the separation of crystalline inclusions of *bt* T84A1 strain from the sporulated culture. There were some differences in the effect of heating and treating with organic reagents on the toxicity of crystal inclusion and crude toxin of the strain T84A1, and toxicity of the former was more stable than that of the latter. The same phenomenon was observed in the case of *bt* var *sotto*. The crystalline inclusions of the strain T84A1 were swelled by the treatment with sodium lauryl sulphate solution. $LD_{50}$ of the crude toxin was 0.8γ/g for silkworm larvae.

**Watanabe T, Iwahana H, Tsutsui and Ogi H (1967). Toxic crystals produced by *Bacillus thuringiensis* T84A1. (II) Action of gut juice of silkworm larvae for toxic crystals *J. Sericult. Sci. Jpn*. 36:16-22.**

Crystalline inclusions of *bt* T84A1 strain were soluble in gut juice of silkworm larvae. It has been assumed that the dissolution of crystalline inclusions with gut juice was not only by alkalinity, but by enzymatic action. Some differences in elution patterns by the use of Sephadex G-200 or DEAE- cellulose between crude toxin and crystalline inclusions degraded with gut juice could be observed. LD50 of purified toxin with Sephadex G-75 was 0.072 γ/g for silkworm larvae. Separation and breakdown of the cell of midgut epithelium and muscle in silkworm larvae were caused by ingestion of purified toxin.

**Kawakita T, Watanabe T and Hayashi K (1967). Studies on the distribution of *enterococcus* in silkworm rearing area. *J. Sericult. Sci. Jpn*. 36:31-38.**

In the silkworm rearing zone of Gifu prefecture, especially in the zone in which the silkworm larvae were infected with flacherie, *enterococcus* was isolated from samples derived from following materials 1) diseased silkworm and dead silkworm in cocoon 2) silkworm feces 3) dust and rubbish from rearing tools and rearing places 4) excrements from livestock and poultry. Then the survey for actual conditions of contamination and the taxonomical study of these isolated strain were carried out.

The selective isolation of *enterococci* in culture medium containing 0.02% sodium azid (EF culture medium) has proved consistently successful. Since 107 strains of *enterococcus* were detected from 151 samples, it has been proved that the distribution of *enterococcus* covers very wide range. After sorting out the isolated *enterococcus*, *Str. faecalis* group and *Str. faecium* group were identified. And number of strains belonging to the latter group was found more abundantly than that of the strains belonging to the latter group was found more abundantly than that of the strains belonging to the former. The strains belonging to *Str. faecium* were detected in abundance in all samples.

**Matsubara F, Kato M, Hayashiya K, Kodama R and Hamamura Y (1967). Aseptic rearing of silkworm with prepared food. *J. Sericult. Sci. Jpn*. 36:39-45.**

Aseptic rearing of silkworm larvae were with prepared food has been carried out to know their condition of development, mortality and quality of the cocoon in comparison with those in the usual rearing with mulberry leaves. The results are summarized as follows:

Silkworm eggs within 48 h of hatching were disinfected, immersing them in 70% ethanol for 20 min and subsequently 0.1% HgCl2 or 2~4% formalin for 20 min. The eggs were washed thoroughly with sterilized water or absolute ethanol and then put in sterilized container.

The newly hatched larvae were put and plugged in the previously sterilized test tubes containing prepared food and incubated at 25°C. The sterilization method of the test tubes and diet was as follows: 100 g of diet was mixed in 160 ml of water and introduced into the tube, plugged with cotton and autoclaved (1.2 kgW/cm$^2$) for 30~40 min. Rearing circumstance through all the stages of asepctical rearing were always under the condition of closed circumstance with high humidity (about 95%) but the silkworm larvae ate and grew well.

The mortality and body weight of the larvae aseptically reared were almost similar to those in the control worms. Cocoon quality, however was somewhat inferior in the aseptical rearing. Cocoon weight and its ratio against the weight (cocoon plus pupa) were 0.22 ~ 0.33 g and 17.5 ~ 19.5% respectively in the aseptical rearing. Those in control worms were 0.43~0.45 g and 20.3~24.0%.

To make the results sure it was required that (1) thus prepared food should be suitable for acceptance of their food by the newly hatched worms, (2) only eggs within 48 h of hatching should be used for axenic work. After hatching, larvae were to be transplanted on the sterilized food (3) the larvae should be transferred on fresh food at least once every week.

In case of aspeptic rearing with prepared food, it is guessed that there is not so bad influence for the growth of silkworm larvae even if they are reared under the above mentioned circumstances during their larvae's period. It is considered that the aseptic rearing is suitable for prepared food rearing and is available for practice in sericulture to obtain aseptic sw (germ free silkworm).

**Utsumi S and Kurisu K (1967). Studies on the inhibition of polyhedrosis in the silkworm, *Bombyx mori* L. (II) The enhancement of incidence of nuclear polyhedrosis by application of methionine to the larva. *J. Sericult. Sci. Jpn*. 36:77-82.**

It has been shown by Y. Kondo that $S^{35}$ of methionine was incorporated to cystathionine, but not to cystine or other members on the pathway of methionine metabolism in the haemolymph of silkworm larvae. And cystathionine was accumulated in the haemolymph of healthy larvae. But the amino acid decreased remarkably in that of nuclear polyhedrosis larvae. As previously reported, taurine and cystine reduced to some extent the incidence of nuclear polyhedrosis of silkworm larvae when these chemicals were orally given with mulberry leaves. In this experiment, injection of taurine into body cavities of the larvae also reduced the incidence of the disease. Methionine enhanced, on the contrary, the incidence of nuclear polyhedrosis of injection into larval body and oral administration. The authors suggested in this report that an attention should be given to the relationship between the incidence of nuclear polyhedrosis and metabolism of S-containing amino acid in the silkworm larvae.

**Ishikawa Y and Asayama T (1967). Studies on the relation between the polyhedroses of the wild insects and the silkworm, *Bombyx mori* L. (V) On the double infection with nuclear polyhedrosis virus in some lepidopterous insects. *J. Sericult. Sci. Jpn*. 36:83-87. [Japanese]**

**Matsubara F (1967). Studies on the diseases in aseptically reared silkworms, *Bombyx mori* L. (III) Occurrence of diseases in silkworms after changing the rearing condition from aseptic to normal. *J. Sericult. Sci. Jpn*. 36:151-158.**

In order to know the nature of the so called latent infection of silkworm polyhedroses, occurrence of the diseases was examined in the silkworm which had been previously reared aseptically with artificial diets and then transferred to normal conditions.

The longer the reared period under normal conditions were, higher was the rate of incidence of nuclear polyhedrosis and this tendency further increased by the cold treatment of larvae, suggesting the latent polyhedroses was activated by the treatment. In the case of complete aseptic rearing, neither polyhedrosis nor any other disease occurred at all.

Therefore, the so called latent infection of polyhedroses seems to occur during the rearing under normal condition and virus diseases are induced by some stresses such as coldness. This means that the aseptic rearing is useful not only for the quarantine of the bacterial disease, but also for the virus diseases such as polyhedroses.

**Matsubara F (1967). Studies on the diseases in aseptically reared silkworms, *Bombyx mori* L. (IV) Influence of some polyhedroses-inducingo chemicals on the aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn*. 36:159-164.**

The effects of some chemicals (ethylenediaminetetraacetic acid, ethylenediamine-tetraacetic acid 2 Na-salt, sodium diethyldithocarbamate, 8-hydroxyquiniline, hydroxylamine and sodium fluoride) on the induction of polyhedroses and the larval development were investigated using silkworm larvae reared aseptically with artificial diets.

When the first four chemicals were fed to the $1^{st}$, $2^{nd}$ and $3^{rd}$ instar larvae respectively, few dead larvae could be found in each lot, but polyhedroses and infectious flacherie were not found at all. When hydroxylamine or sodium fluoride were fed to larvae of each stage, they mostly died within 4~9 days after feeding, without incidence of polyhedroses.

It is considered therefore that, the polyhedrosis virus was not present in the larval body under aseptic condition, even if some physiological abnormalities in larvae were induced by fed chemicals.

**Kobara R, Aruga H and Watanabe H (1967). Effect of larval growth on the susceptibility of silkworm, *Bombyx mori* L., to a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn*. 36:165-168.**

The present study was designed to clarify the change of response of the silkworm of resistant and susceptible strains to the infection of a cytoplasmic polyhedrosis virus during the fourth instar. The experimental results revealed that susceptibilities of the both strains were markedly varied with larval growth. The susceptibility was high in the early stage and much lower in the middle stage, and then increased upto prior to the moult. When the larvae just after ecdysis were fed for 5, 10, 20 and 40 h on mulberry leaves smeared with various concentrations of the virus polyhedron suspension, the more the virus at each concentration was ingested as feeding went on, the higher the rate of infection was observed, but no further increase was detectable at feeding for 20~40 h. Thus the depressed infection in the virus fed larvae for the longer period might be caused by the low larval response which had varied with growth during the virus feeding.

**Tanaka S and Aruga H (1967). Interference between the midgut nuclear-polyhedrosis viruses and the cytoplasmic-polyhedrosis viruses in the silkworm *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 36:169-176.**

When both the NPV and CPV were fed together to the 3$^{rd}$ instar larvae of the silkworm, and the concentration of polyhedra of both viruses was not equal, the two viruses interfered with each other. In the case of mixed virus infections in which both polyhedra were formed in the midgut of one larva, two types of polyhedra were not observed in each cylindrical cell.

The midgut nuclear polyhedrosis viruses interfered with the infection by the cytoplasmic polyhedrosis virus when the midgut nuclear polyhedrosis viruses were fed to the second or the third instar larvae and the cytoplasmic polyhedrosis viruses were fed after one interval of 1 or 4 days.

The extent of the interference varied with the conc. of the two viruses and the interference between the two viruses was remarkable when the relative concentration of the midgut nuclear polyhedrosis virus was greater than that of cytoplasmic polyhedrosis virus.

**Tanaka S (1967)/ Oviposition of the moth of silkworm, *Bombyx mori* L., to midgut nuclear-polyhedrosis virus infection in the larval stage. *J. Sericult. Sci. Jpn*. 36:177-182. [Japanese]**

**Aizawa K (1967). Mode of multiplication of the Nuclear Polyhedrosis virus of the silkworm. *J. Sericult. Sci. Jpn*. 36:327-332. [English]**

Virus multiplication in the early period of infection was analyzed in more detail by using alkaline homogenated tissues of infected pupae and it was found that the decreasing phase continued for 4 hours and the eclipse appeared 4~6 hours after infection. A peak in virus infectivity titer, which is considered to be based on a release of multiplied virus, occurred 6~8 hours after infection. Then the titer decreased and 10 h after infection the logarithmic increasing phase appeared. The free infectious DNA does not exist in the liquid phase of infected hemolymph. There may be many factors controlling virus infection and multiplication. One is the antiviral action of insect gut juice. The supernatant of infected hemolymph was inoculated either subcutaneously or orally into fourth instar larvae. It was evident that the lethal dose was lower by intrahemocoelic infection than by oral infection and furthermore, the gut juice inactivated the virus *in vitro* (Aizawa, 1962).

**Tanada & (1967). Effect of High Temperatures on the Resistance of Insects to Infectious Diseases. *J. Sericult. Sci. Jpn*. 36:333-340. [English]**

The resistance of insects at high temperatures has been established in all of the four major groups of insect viruses (the nucleopolyhedrosis, the cytoplasmic polyhedrosis, the granulosis and the non inclusion viruses). High temperature may not only affect the development of virus particles, but it may also stimulate or cause the formation of immune host reactions.

**Yamafuji K (1967). Isolation of polyhedral pre-viral Deoxyribonucleic Acid from Healthy Silkworm. *J. Sericult. Sci. Jpn*. 36:341-345. [English]**

DNA was prepared from both silkworm cells and viral particles. Although it had been believed that the preparation of high molecular DNA from insects was almost impossible, we are now able to obtain a fibrous nucleic acid by our improved method with sodium dodycylsulphate. Pure DNA fibre was also prepared in a similar way from viral polyhedra. Chemical analysis was then performed for both DNA preparations. The results disclosed that the base composition of cellular and polyhedral nucleic acids is nearly the same.

**Watanabe H (1967). Autoradiographic studies on the nucleic-acid synthesis in the midgut of the silkworm, *Bombyx mori* L., under normal and virus infected conditions. *J. Sericult. Sci. Jpn*. 36:371-380.**

The patterns of nucleic acid synthesis activity within the midgut cells of healthy silkworm larvae and of larvae infected with the CPV as well as flacherie virus were demonstrated by means of autoradiography with titrated nucleic acid precursors.

Autoradiographs with $^{3}$H-thymidine revealed no essential difference in the pattern of DNA synthesis between healthy and cytoplasmic polyhedrosis midguts, and only a few cells incorporated the labeled material into their nuclei. At the late stage of the virus infection, however, when some infected midgut cells eventually degenerated, there was a slight increase in the nuclear label in the newly regenerated cells. On the other hand, in the nuclear polyhedrosis midgut, hypertrophic nuclei of cylindrical cells as well as goblet cells were densely labeled with $^{3}$H-thymidine without accompanying any formation of polyhedra. In the midgut infected with flacherie virus, most of the cylindrical cells incorporated much of $^{3}$H-thymidine into their nuclei during the course of the disease, while no detectable radioactivity was seen on the goblet cells.

At five hours after injection of $^{3}$H-uridine, the healthy cells generally incorporated the labeled material into cytoplasmic RNA and partly into nuclear RNA, whereas in the cytoplasmic polyhedrosis midgut the diseased cells incorporated much of the labeled uridine into nuclear RNA and some into cytoplasmic RNA. In the nuclei of the virus infected cells, the nuclear label appeared most densely over the nucleoli. In the nuclear polyhedrosis midgut, however, the incorporation of $^{3}$H-uridine into both nuclear and cytoplasmic RNA was slightly larger than that in the healthy one. In the early stage of flacherie-virus infection, both cylindrical and goblet cells incorporated a great amount of $^{3}$H-uridine into their nuclei and some into the cytoplasm, while at the late stage of infection, degenerating goblet cells still actively incorporated the labeled material into their nuclei and most of the label seemed to diffuse to be accumulated in the cytoplasm around the goblet.

**Watanabe H, Aruga H and Tanaka S (1967). Autoradiographic studies on the nucleic-acid synthesis in the midgut of the silkworm, *Bombyx mori* L., infected with midgut-nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn*. 36:381-387.**

The pattern of nucleic-acid synthetic activity within the midgut cells of the silkworm larva infected with midgut nuclear-polyhedrosis virus was demonstrated in this paper by means of autoradiography with titrated nucleic acid precursors.

Autoradiographs of the diseased midgut treated with $^{3}$H-uridine revealed that at the early stage of infection a high grain density was found over the nucleus of the cylindrical cell, while the small amount of grain was scattered all over the cytoplasm. In the virus infected cell, the nuclear label appeared most densely over the nucleolus, suggesting a large amount of RNA was synthesized there. At the later stage of infection when large hexahedron polyhedra develop in the nucleus of cylindrical cell, most of the autoradiographic grains were seen around the polyhedra.

Authoradiographs with 3H-thymidine suggested that no essential difference in the pattern of DNA synthesis existed between the healthy and the virus infected midguts. Only a few regenerative as well as cylindrical cells incorporated $^{3}$H-thymidine into their nuclei. At the later stage of the virus infection, however, when most of the diseased cylindrical cells were eventually discharged into the gut lumen, autoradiographs of the midgut showed that the uptake of $^{3}$H-thymidine into the nucleus extremely increased in the regenerative cells and the newly developed cylindrical cells as well. This acceleration of DNA synthesis apparently has no direct relation with the virus multiplication but is concerned with the regenerative development of cells. Although in the midgut nuclear polyhedrosis polyhedra only develop in the nuclei of midgut epithelial cells, the pattern of nucleic acid synthesis in the virus infected cell is much similar to that in the cytoplasmic polyhedrosis which has been reported previously.

**Aoki J (1967). Physiological function of hyphal bodies in insect causal fungus, *Isaria fumoso-rosea*. *J. Sericult. Sci. Jpn*. 36:388-394.**

In Japan, it has been generally recognized that hyphal bodies are termed cylindrical spores. The purpose of the following work is to make clear whether the hyphal bodies are of vegetative or reproductive nature by investigating the physiological function of hyphal bodies with the help of conidiospores. The author observed the phase of production and development of hyphal bodies and conidiophores (in place of conidiospores) with *Isaria fumoso-rosea* growth on synthetic median slide cultures, prepared with different concentrations of glucose content, ie., 4, 2, 1, 0.5 and 0.25%. It was found that the formation of hyphal bodies as well as growth of hyphae increased in media of higher glucose content. On the other hand, the formation of conidiophores increased with lower concentration of glucose in early stage of the culture. With the intake of glucose by the fungus in media, hyphal bodies sprouted and developed subsequently to hyphae and conidiophores were remarkably formed. Hyphal bodied, developing into hyphae, disappeared through either of the following way; the one that their protoplasm was transferred into newly sprouted hyphae from hyphal bodies, becoming emptied cells, like a skeleton, and the other one was that the protoplasm was reabsorbed by mother hyphae, becoming `ghost cells'. On the basis of these observations, it seemed reasonable to assume that hyphal bodies had similar nature to hyphae, having in addition temporary storing function of the nutritious substance.

**Kobayashi M, Yamaguchi S and Agatsuma T (1967). Studies on the occurring time of nuclear-polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn*. 36:395-399.**

In order to obtain some information concerning the mechanism of resistance to the *per os* infection by nuclear polyhedrosis virus, the latent period and further more the hormonal effect upon the attack rate of the virus were investigated in the silkworm larvae. The results are as follows:

When the larvae wee inoculated with the NPV immediately after the 3rd or 4th ecdysis; a) In the case of 108 POB/ml, the disease appeared in all larvae before or after the next moulting period. b) In the case of 107 POB/ml or 106 POB/ml, the disease appeared at some intervals, only during the molting or in matured larvae, and c) the occurrence of the disease was recognized to be repressive while the larvae were eating mulberry leaves.

When the larvae were inoculated with the virus of nearly $ED_{50}$ in the middle of the 4th instar, the disease appeared in the next instar in a few of them, but appeared in most of them before or after the mature period. In some strains and F1 hybrids of relative resistance to the virus infection, some of the larvae survived in the next molting period and acquired the disease in the later periods. In order to analyze the causal factor of the occurrence of the disease, an ether soluble component extracted from corpora allata was injected into the larvae after the virus inoculation. This extract solution showed the tendency to increase the attack rate of the disease.

## Vol. 37 1968

**Kobayashi H and Ayuzawa C (1968). On the complement-fixation test of the infectious flacherie virus of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:13-16.**

The complement fixation test of the infectious flacherie virus of the silkworm was carried out. The antisera against the virus were prepared in rabbits by injection of the virus which had been obtained by ultracentrifugation or equilibrium density gradient method using CsCl. The complement fixation reaction was observed in these antisera, though at a low titer of the antisera, and may be used as one of the methods to calculate the titer of the antisera against the infectious flacherie virus.

**Ohshima K (1968). Experimental design on a sampling inspection method of pebrine control by utilizing the nature characterized by early eclosion of diseased silkworms with pebrine of a lot. V. Relationship between pebrine disease of silkworm moths and their eclosion dates. *J. Sericult. Sci. Jpn.* 37:17-26.**

The percentage of the diseased female moths of silkworm always diminished with high speed in accordance with retardation of eclosion date irrespective of the difference of maturation days of larvae and their combination as those of the previous reports in both cases. In the latter, however, one of the 3 experiments showed no such symptom. It may be due to the great decrease of host nutrients necessary for metamorphosis. This assumption was derived from the fact that during metamorphosis, the multiplication grade of parasites in one experiment was exceedingly superior to that of the two others. In the case of the diseased male moths, their diminution velocity and their irregularity were also almost the same as those of the previous reports in the former and the latter mentioned above. However, the two others normally diminished their infection percentages with delayed eclosion dates. In addition, some information requiring the attention of silkworm egg producers were reported on the pebrine disease occurring in the neighboring areas. The case of high infection percentages of moths about 47-70% and weak worms eclosed about 31-8%.

**Yamaguchi K (1968). Studies on the midgut nuclear polyhedrosis in the silkworm, *Bombyx mori* L. (I) The formation site and some nature of the polyhedra. *J. Sericult. Sci. Jpn.* 37:34-42.**

The present paper deals with results on a new polyhedrosis virus forming polyhedra in the nucleus of midgut epithelium in the silkworm. Histo-pathological studies showed that polyhedra were mainly formed in the nuclei of the cylindrical cells of midgut, but in a few cases they were formed in both the cytoplasm and nucleus of the same cell. The polyhedra are hexahedral and are from 2.5 to 22.5μ in diameter. They were stained with bromophenol blue, methyl orange, fuchsin, aniline red, orange G, phloxine or easin after being hydrolyzed in 1N HCl at 60°C for 5 min.

Interference was observed between the midgut nuclear polyhedrosis virus mentioned above and the cytoplasmic polyhedrosis virus in the midgut epithelium, when two viruses were fed to a

larva at the same time, either type of polyhedra was formed in a cylindrical cell, but both kinds of polyhedra were not observed in the same cell. The larva was infected when a newly hatched larva was fed only one large polyhedron (about 20μ in diameter).

**Yamada T, Yamazaki H and Kobayashi A (1968). Effect of wood ash and some manures on the inactivation of the flacherie virus. *J. Sericult. Sci. Jpn.* 37:66-68. [Japanese]**

**Matsubara F (1968). Studies on the diseases of the aseptically reared silkworms, *Bombxy mori* L. (V) Induction of the nuclear polyhedrosis by the feeding of certain chemical to the silkworm after transfer from asepctic to normal rearing condition. *J. Sericult. Sci. Jpn.* 37:137-143.**

The present experiment was undertaken in order to study the condition in which the NPV can be induced by feeding certain chemicals. The following chemicals such as ehtylenediamine tetraacetic acid (EDTA), Disodium ethylenediaminetetraacetate, sodium diethyldithiocarbamate, 8-hydroxyquinoline, hydroxylamine and sodium fluoride were fed to the 5$^{th}$ stage silkworm larvae, which were previously reared aseptically and then transferred to usual open condition. The results are summarized as follows:

Nuclear polyhedrosis was induced by feeding above chemicals in only fewer cases (5-35%) when the usual open rearing was performed for longer period (from 2ne to 5$^{th}$ larval stage). Cytoplasmic polyhedrosis was not induced in such a condition. Both polyhedroses were not found in the silkworm reared aseptically throughout the larval period of those reared in open condition for only the 5$^{th}$ larval period. No difference was found in the rate of nuclear polyhedrosis induction between the larval groups fed with different kinds of diets (mulberry or artificial). Among six different chemicals, hydroxylamine and sodium fluoride were the most effective for induction of nuclear polyhedrosis. The feeding of these two chemicals resulted in the increase of the larval mortality due to the flacherie. Some discussions have been made in the present paper about the nature of the induction of nuclear polyhedrosis by the feeding of chemicals.

**Yamazaki H, Yamada T and Kobayashi A (1968). Occurrence of the non-moulting tiny silkworms caused by inoculation of the flacherie virus. *J. Sericult. Sci. Jpn.* 37:155-156. [Japanese]**

**Utsumi S, Kitano M, Onishi M, Kurisu K, Yamaguchi C and Ichikawa Y (1968). Application of β-propiolactone (PL) on the rearing of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:207-212.**

It was previously reported by the authors that β-propiolactone (PL) was effective as a disinfectant to the pathogenic microorganisms of the silkworm. The larvae were rather resistant to the orally administrated PL because of the rapid decomposition of this chemical in the highly alkaline digestive fluid. Thus it was supposed that PL could be used as a disinfectant for the rearing of the silkworm larvae.

To know the practical problem of the utilization of PL to prevent the silkworm diseases during the rearing period, 0, 1, 1.25 and 2.5% PL solutions were sprayed respectively once or twice a day on the rearing seats of the 4$^{th}$ or 5$^{th}$ instar larvae. And the effect of such spray on the growth of the larvae and also on the rate of healthy pupae were investigated. A marked preventing effect of PL on the occurrence of the silkworm disease was recognized when 2.5% solution was sprayed once a day, and the rate of the healthy pupae clearly increased in this lot. The harmful effect on the physiology of the silkworm, however, was found in the case of PL spraying at low environmental temperature or with the excessive use.

**Watanabe M, Funada T, Kubota A, Kawakami T, Aizawa K, Kita S and Mastsuo T (1968). Effect of some disinfectants on the flacherie virus of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:213-218.**

Effect of some disinfectants on the flacherie virus of the silkworm was examined. Chlorinated lime was the most effective to inactivate the virus among the disinfectants tested. The virus was inactivated completely with a 0.5% solution for 10 min. at 10°C. Hydrochloric acid (15%) inactivated the virus effectively after 60 min at 24°C, 6 min at 48°c and 30 min at 10°c but the virus remained viable after 15 min at 10°C. Cresol soap (5%), benzalconium chloride (1%) and Dysen stainless (0.1% zinc ethylenebisdithio-2-ammoniac carbamate) did not inactivate the virus after 2 h at 24°C.

**Funada T (1968). Genetic resistance of the silkworm, *Bombyx mori* L., to an infection of a flacherie virus. *J. Sericult. Sci. Jpn.* 37:281-287.**

Genetic resistance of the silkworm to the flacherie-virus infection was studied using several Japanese and Chinee races as well as their hybrids. The results obtained are summarized as follows:

Resistance to the viral infection is a recessive genetic character which is probably controlled by a major gene. Hybrid vigour or heterosis in the resistance could not be entirely observed among the tested hybrids. It was also found that the selection of a resistant strain could be readily accomplished by the batch selection method for several generations.

**Aoki J and Chigusa K (1968). Studies on the nutrition and metabolism of pathogenic fungi in muscardine disease. (I) Nitrogen utilization by synthetic media of *Beauveria bassiana, Isaria farinose* and *I. fumoso-rosea*. *J. Sericult. Sci. Jpn.* 37:288-294.**

The growth responses of *Beauveria bassiana, Isaria farinose* and *Isaria fumoso-rosea* to different nitrogen sources were studied. Experiments showed that glutamic acid, aspartic acid among amino-nitrogens, ammonium oxalate, ammonium citrate, and ammonium tartrate among other nitrgens induced better growth on these organisms. Other amino acids affected differently the growth of each organism, while inorganic nitrogens scarcely showed diference in the suitability to the growth of each organism. Glutamic acid, aspartic acid and ornithine were commonly found in culture filtrates of *B. bassiana* and *I. fumoso-rosea*. Mycelia contained 6 amino acids in the former, 8 amino acids in the latter, including glutamic acid and aspartic acid.

**Takizawa Y and Iizuka T (1968). The anaerobic bacterial flora in the gut of larvae of the silkworm, *Bombyx mori* L. (I) The relation between media and the numbers of living cells. *J. Sericult. Sci. Jpn.* 37:295-305.**

The normal aerobic bacterial flora in the gut of larvae of the silkworm, *B.m.* was examined by using some selective media. A large number of different bacteria species was isolated from the midgut and an attempt to identify all the microorganisms isolated was made.

Living cells were rapidly increased on the first day after feeding and they were also isolated continuously until the larvae reached the $5^{th}$ instar. As a single medium to isolate the most numbers of living cells, nutrient agar had a good effect and to isolate using with PEA medium which is a selective medium for gram positive *enterococci*, also had the best effect. The composition of the aerobic normal bacterial flora from midgut was identified as *Micrococcaceae, Bacillaceae, Brevibacteriaceae, Lactobacillaceae, Enterobacteriaceae, Psuedomonadaceae* and *Achromaobacteriaceae*. The following predominant microorganisms were classified by considering the frequency of occurrence of them (more than 20%) and the numbers of living cells (more than $10^6$/ml per larva by summing the mean numbers of each day in the $5^{th}$ instar larvae).

They are *Staphylococcus epidermidis, Streptococcus* spp., *Bacillus cereus, Staphylococcus aureus, Klebsciella ozaenae, Alcaligenesmetalcaligenes, Aerobacter cloacae, Pseudomonas faimontensis, Psudomonas riboglavina* and *Achromaobacter parvulus*. The gram negative strains in the normal flora wre generally isolated on the first day or second day after feeding and the gram positive strains were constantly isolated from 5$^{th}$ instar larvae.

**Kobayashi H, Sato F and Ayuzawa C (1968). On the disinfecting ability of the mixture of bleaching powder and formalin. *J. Sericult. Sci. Jpn.* 37:311-318. [Japanese]**

**Watanabe H, Kobara R and Hosaka M (1968). Electrophoretic separation of the haemolymph proteins in the silkworm, *Bombyx mori* L., infected with the nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 37:319-322.**

By means of thin layer electrophoresis in agarose gel, haemolymph proteins of the healthy silkworm larvae and of the nuclear polyhedrosis infected larvae were studied. Seven protein fractions, including three main fractions (B, E and G) moving towards the anode were generally detected in the healthy larvae in the 5$^{th}$ instar. In the larvae at the earlier stage of infection when the small polyhedra were visible in the cells of fat body, the haemolymph protein patterns were essentially the same as those of the healthy larvae. In a moe advanced stage of infection, the haemolymph of the diseased larvae contained less protein, particularly of the medially mobile fraction E and the fraction C with lower mobility, than did the healthy larvae. In some cases, the fraction E in the diseased haemolymph was lowered in mobility. Furthermore, in the milky white haemolymph of heavily diseased larvae in which a large number of free polyhedra from disrupted fat body were floating, all protein fractions were greatly reduced. Inasmuch as the fat body of the sw larvae is the center of haemolymph protein synthesis and also is one of the most susceptible tissues to the nuclear polyhedrosis virus, hypoproteinemia observed in the electrophoresis is probably a direct consequence of the viral lesions in the fat body.

**Iizuka T and Takizawa Y (1968). A method for counting bacterial cells in the midgut of the larvae of silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:333-336.**

A number of bacterial cells in the midgut of the larvae of silkworm have been microscopically counted on the smear samples. The preparations were obtained by aseptically dissecting the midgut of larvae, 0.01 ml of preparations was taken out with a micropipette and smeared with the needle on a glass slide, the space measuring 1 cm$^2$. Glass slides were dried for 5 min on a water bath heated at 50°C and then they were instantly dipped into Newmann's staining solution. The result of microscopic observation in these smear samples, morphological characteristics of bacterial cells could be slightly distinguished. By using the same preparations and isolating bacteria with nutrient agar and PEA medium, viable cells were counted. Numbers of bacterial cells were from $10^6$ to $10^7$ ml and numbers of viable cells were less than the former. There was a large difference between the numbers of bacterial cells on samples and that of viable cells on isolating media.

**Watanabe H (1968). Pathogenic changes in the faeces from the silkworm, *Bombyx mori* L., after *per oral* inoculation with a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 37:385-389.**

Pathogenicities in the feces from the virus ingested silkworm were studied in this paper. The virus employed in this study was a CPV which was occluded in hexahedral polyhedra. The virus was administered perorally and individually to the larvae just after the 3$^{rd}$ ecdysis by feeding them for 18 h on mulberry leaves contaminated with a high concentration of the virus polyhedra. Then, each larva was reared separately on clean leaves. Faeces from each larva were collected at 24 h intervals and were stored in the deep freezer until ready to use. On the9th day after the virus administration, when most tested larvae were in the 5$^{th}$ instar, all of them were dissected and their

contents were examined under the microscope to determine the infected larvae from the uninfected ones. The result was that, some tested larvae were heavily infected and the others were healthy without suffering from the ingested virus disease.

In the following step, stored feces of each lot were macerated with 0.9% NaCl solution and centrifuged. Te supernatant was perorally administered to the 1st instar larvae to estimate the pathogenicity in the faeces, while the debris was examined microscopically to ascertain the presence of polyhedra.

The results in the non-diseased larvae indicated that a high pathogenicity was generally detected in the faeces for about one day after the virus administration and subsequently undetectable. However, the faeces obtained shortly before or after the 4th moult were again markedly pathogenic. The pathogenicity in the faeces soon after feeding of the virus was probably due to the excretion of the ingested viruses. Furthermore, it was highly probable that the second peak of pathogenicity noted in the faeces obtained before or after the 4th moult can be attributed to the ingested viruses which had been retained in the peritropic membrane and excreted with it around the time of moult when the membrane was known to be renewed. No polyhedra were always detected in the faeces from the non diseased larvae.

In the faeces from diseased larvae, the pathogenicity was clearly high at the time of the virus administration and then undetectable for a while. But the pathogenicity again appeared in the faeces about 3 to 5 days after the administration, accompanying with the excretion of some irregular shaped polyhedra. Furthermore, as the disease progressed, the faecal pathogenicity increased. At the late stage of the disease, most of the facese showed whitish colour containing a large number of hexagonal polyhedra. Thus, the secondarily appeared pathogenicity in the faeces from the diseased larvae resulted from the excretion of a large number of free viruses which had been produced in the infected midgut. The increase in the number of regular shaped polyhedra in the faeces from the heavily diseased larvae might be a reflection that the pH of gut juice had been reduced as the disease progressed.

**Miyajima S and Kawase S (1968). Effect of a high temperature on the incidence of cytoplasmic-polyhedrosis of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:390-394.**

In this paper, the effect of high temp on the incidence of cytoplasmic polyhedrosis of the silkworm is described. Results obtained are summarized below.

When newly hatched silkworm larvae were reared at high temp (34°C) after being inoculated *per os* with CPV or polyhedron suspension including antibiotics (penicillin, 500 units/ml in final concentration), the rate of incidence of the polyhedrosis was lowered at high temperature as compared with the case at 25°C. In relation to the decreased incidence of the polyhedrosis by interaction between high temp and the dosage of polyhedra, per oral administration of lower dose (106 polyhedra/ml) showed more clear heat therapy effect than that of higher dose (108 polyhedra/ml). Similar results were obtained when the virus was injected into 3rd instar larvae. The silkworm larvae of the first day of 5th instar were reared at 25, 30 or 40°c after the virus injection, and the number of polyhedra formed in the midgut epithelium of the infected larvae was surveyed every 24 h thereafter. The number and the rate of increase of polyhdra formed in the midgut of the silkworm was the highest at 30°C, but lowest when reared at 34°C. From these results, it may be concluded that high temp such as 34°C suppresses the incidence of cytoplasmic polyhedrosis of the silkworm.

**Ayuzawa C, Furuta Y, Kodama R and Nakasuji Y (1968). On the synergism between the viruses and the bacteria in the development of flacherie of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:395-402.**

The present experiments were carried out to clarify the synergism between the viruses and the bacteria in the development of flacherie of the silkworm, by aseptic and individual rearing.

The pathogens used were infectious flacherie virus and *Streptococcus faecalis-Str. Faecium intermediate* G-27. The reasons why this bacterial strain was used were as follows: 1) this strain was isolated from the diseased silkworm 2) this strain was supposed to be spread widely in Japan 3) the pathogenicity of this strain for the silkworm was relatively low 4) much bacteriological information on this strain was available 5) this strain was not a pathogen of the septicemia in the silkworm. To avoid the propagation of the strain tested in a diet, the time of the inoculation by the diet infected with the bacteria was limited to 4 h and the larvae were transferred into new tubes containing aseptic diet, and the transfer was repeated daily. Inoculation of the virus was carried out at the $3^{rd}$, $4^{th}$ and $5^{th}$ instars, with bacteria at the $4^{th}$ and $5^{th}$ instar, and with the pathogens in both of these combinations.

The mortality ranged from zero to 20% when single pathogen was used, but when challenged with both pathogens, the mortality became much higher than those obtained with single pathogen, ranging as high as from 60 to 100%. Especially, the mortality increased when the larvae were challenged with the virus in the $3^{rd}$ instar or $4^{th}$ instar and subsequently with the bacterium at the $5^{th}$ instar. As far as the present study was concerned, the synergistic association between the virus and the bacteria was clearly observed. The multiplication or propagation of the pathogens in the diseased larvae was investigated by bioassay or colony counting method. The number of cells of the bacteria per larva was over $10^8$, on the other hand, infective titer of this virus was not necessarily high.

**Ohshima K (1968). Experimental design on a sampling inspection method of pebrine control by utilizing the nature characterized by early eclosion of diseased silkworms of a lot. VI. Statistical investigation on the lowering rate of the percentage of diseased female moths with retardation of eclosion dates and on a device for improving pebrine control. *J. Sericult. Sci. Jpn.* 37:408-419.**

The relationship between the percentage of pebrine disease of cumulative number of daily eclosed female moths and their daily decreasing percentage makes a regression line. The ratio of the disease percentage of the cumulative number of moths early eclosed to that of the same lot is influenced by the difference of lowering speed of its percentage and the eclosion days. However, it tends to become 1 at the end of eclosion date irrespective of their different lots. It may be therefore possible to obtain a number which may at the same time becomes statistically equivalent to the variances among different lots and to those of respective lots by increasing the first cumulative number of moths little by little. Based on this conception, the cumulative number of moths early eclosed which satisfies the above condition was experimentally obtained by calculating the variance distribution table composed of the ratio of each first cumulative disease percent of moths to that of the lot itself. That is, the required minimum number is 140 in a lot; lot tolerance percent defective is 1.16% and when the ratio of disease % of 140 moths early eclosed of a lot to 1.16 % is 3 or less, the silkworm eggs of that lot is passable as seeds for common silkworm rearers. However, as 1.16 is an arbitrary constant for common hibernating eggs which is temporarily adopted by the author's professional knowledge, it cannot be used at present. Only the following two cases can safely be adopted for practical use immediately.

The case of extremely low percentage of Pebrine: When the ratio of disease percentage of 30 moths early eclosed of a lot to 1.16% is 2 or less, all the eggs of that lot can safely be passed as excellent seeds for sericulture. The case of high percentage of Pebrine: When the ratio of disease percent of 30 moths early eclosed of a lot to 1.16% is 16 or higher, all the eggs must be sacrified as intolerant seeds for sericulture. More than 80% of the whole lots, irrespective of their kinds, whether they are passable or not can be determined by the above two methods, as the incidence of pebrine disease is now very less in Japan. When the ratio is a little higher than

tolerance limit 3, when inspected 140 moths early eclosed of a lot, its great part can be passed by rejecting the early eclosed moths which exceed. This method, however, can only be adopted after a lot tolerance percent defective is exactly determined.

**Aruga H and Tanaka S (1968). Effect of high temperature on the resistance of silkworm, *Bombyx mori* L. to flacherie-virus disease. *J. Sericult. Sci. Jpn.* 37:441-444.**

Studies on the effect of high temperature on the flacherie virus of the silkworm, *Bombyx mori* L., were conducted by using a few resistant and susceptible F1 hybrids in spring and summer seasons. The newly hatched larvae or the second instar larvae were reared at 32°C and 25°C after the inoculation of the flacherie virus of several dosages. It was shown than when the silkworm larvae were reared at high temperature of a few days after inoculation, they were able to resist the infection by the flacherie virus.

**Yamaguchi K (1968). Studies on the midgut-nuclear polyhedrosis in the silkworm, *Bombyx mori* L. II. Temperature effects on the formation of polyhedra. *J. Sericult. Sci. Jpn.* 37:462-470.**

The study was conducted to find out the effect of high temperature on the formation of polyhedra in the silkworm larvae infected with the midgut nuclear polyhedrosis virus. The larvae were reared at various temperatures after virus inoculation and polyhedra formed in the midgut epithelium of diseased larvae were observed under a light microscope. The polyhedra were normally tetragonal in outline when the silkworms were reared at 18°C and 25°C but showed irregular forms at 30°c and were not formed at 35°C. Histological studies showed abnormal polyhedra showing several irregular forms and large deviation was noted in the size of polyhedra formed in the nuclei of the cylindrical cells of midgut. The abnormal polyhedra were stained with bromophenol blue, orange G, fuchsin, Phloxine methyl orange eosin, aniline red of Heidenhain's iron alum hematoxylin after being treated with 1N HCl at 60°C.

**Ishikawa Y and Miyajima S (1968). Infection between infectious flacherie virus and some bacteria in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 37:471-476.**

In order to clarify the interaction between infectious flacherie virus and some bacteria in the silkworm, *Bombyx mori*, the virus and two kinds of bacteria, *ie*, *Streptococcus* and *Serratia* isolated from flacherie larvae were used in this experiment. The results obtained are as follows;

When silkworm larvae were inoculated *per os* with both flacherie virus and bacteria, the incidence of flacherie was higher and latent period was shorter than in the case of virus inoculation alone. Silkworm larvae were more susceptible to the virus infection, when administered with it before or after inoculation with bacteria. On the other hand, this phenomenon could not be noted in case of inoculation of a mixture of the virus and bacteria (especially *Serratia*) inactivated by boiling to silkworm larvae. When the larvae were inoculated with only these bacteria, the occurrence of the disease was not recognized.

**Kodama R and Nakasuji Y (1968). Bacteria isolated from silkworm larvae. I. The pathogenic effects of two isolates on aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 37:477-482.**

Investigation was made on the pathogenic effects of two bacterial strains isolated from silkworm larvae, E-5 and E15, employing healthy silkworm larvae in the 5$^{th}$ instar, which were reared aseptically on an artificial diet. The results obtained were:

When each strain was inoculated to larvae by feeding, mortality eventually reached 100%. The symptoms, however, varied with the species, that is, larvae infected with the strain E-5

shrinked following diarrhea, while those infected with the strain E-15 became generally numb during the next several days, although they went on with their growth until they succumbed. Death of larvae was hastened by mixed infection of both strains, exhibiting a synergistic effect between the pathogenicity of the strain E-5 and that of the strain E-15. The strain E-5 was capable of growing in media of relatively high pH values compared with the strain E-15.

**Watanabe S (1968). An observation of the spore of *Nosema bombycis* NAGELI under the interface phase contrast microscope. *J. Sericult. Sci. Jpn.* 37:491-497.**

Observation of the spore *Nosema bombycis* NAGELI by means of an interference phase contrast microscope revealed that there are remarkable differences in phase contrast colour between spores and backgrounds or debris and that the spores are easily detected by their sizes and figures. In higher magnification, the spores showed more distinct differences in colour and they have a characteristic component in the central part.

Detection of the spore of *N.b*. is easy when observed in sensitive colour keeping the angle of analyzer at 45° and polarizer at 120° or in the blue region of analyzer at 45° and polarizer at 130°. Under monochromatic observation, the phase differences between the spores and backgrounds showed 73.4mμ and those between spores and debris were assumed to be about 26.4 mμ. The spores of *N.b*., the conidiums of muscardine of silkworm and the pollen pellets of Indian corn could be distinguished from each other by their interference colors, figures and sizes. They are more easily distinguished under higher magnification by the existence of an inner component in the spores. When the interference phase contrast microscope is used in the examination of female moth for parasites, the field is more clear and the colour contrast is more distinct than the ordinary light microscope. Therefore, it is concluded that the detection of spore is remarkably increased by this method.

## Vol. 38 1969

**Tanaka S (1969). Comparison of the mode of multiplication of flacherie virus in the silkworm strains having different susceptibilities. *J. Sericult. Sci. Jpn.* 38:21-27.**

The rate of multiplication of flacherie virus in he larvae of several resistant and susceptible strains of the silkworm, *Bombyx mori* L., was investigated. The results are as follows:

The flacherie virus was fed to the first instar and 3$^{rd}$ instar larvae of seven silkworm strains, *ie*, N124, Shungetsu, C115, C124, Okusa, Daizo and N124 x C124 in order to compare their susceptibilities. The result showed that N124, Okusa and N124 x C124 were susceptible, while Daizo was highly resistant. The amount of virus multiplied in the infected larvae was investigated at 24 h, 48 h and 72 h after the virus inoculation. At 72 h, a parallel relation between the resistance and the rate of multiplication of flacherie virus was observed. The results showed that the rate of multiplication was low in resistant strains while high in susceptible strains.

The rate of multiplication of the flacherie virus was investigated in the highly susceptible N124 and Daizo, which was highly resistant. In a few experiments carried out in the spring and summer rearing seasons, the rate of infection of the flacherie virus in N124 was 100% in both seasons, while in Daizo, it was 7% in spring and 41% in summer seasons. Thus Daizo was proved to be highly resistant to the flacherie virus, whereas the virus multiplied actively in the larval bodies of the resistant strain Daizo. No symptoms could be observed in some larvae of Daizo in which considerably large amount of virus multiplied.

**Matsubara F and Hayashiya K (1969). The susceptibility to the infection with nuclear-polyhedrosis virus in the silkworm reared on artificial diet. *J. Sericult. Sci. Jpn.* 38:43-48.**

Difference in the susceptibility to the infection with nuclear polyhedrosis virus between silkworm larvae reared on mulberry leaves and artificial diet was investigated. The results were as follows:

The larvae reared on the artificial diet were more susceptible to the oral infection with nuclear polyhedrosis virus than those reared on mulberry leaves. No difference in the susceptibility was observed, when the virus were given subcutaneously to the both larvae. Anti-viral activity of the digestive juice from the larvae reared on artificial diet was lower than that of larvae reared on mulberry leaves. It was suggested that high susceptibility to nuclear polyhedrosis virus in the larvae reared on artificial diet is referred in part to the low anti-viral activity.

**Iwashita Y and Kanke E (1969). Histopathological diagnosis of diseased larvae infected with flacherie virus of silkworm, *Bombyx mori* LINNAEUS. *J. Sericult. Sci. Jpn.* 38:64-70.**

Histopathological observation on the silkworm larvae infected with the flacherie virus revealed the formation of two types of cytoplasmic inclusion bodies, A and B types, in the midgut epithelium. The A type inclusion body seems to be formed by the endogeneous reaction of the host cell, whereas the B type inclusion body may be produced by the degeneration and danaturation of the goblet cell. These inclusion bodies were not entirely formed in the midgut epithelium of the flacherie larvae infected with bacteria.

Hisotological staining of a part of the midgut epithelium of flacherie larva with pyronine methyl green by the smear method or the super vital staining method was effective to determine the presence of inclusion bodies. The histological test was diagnostically applied to flacherie larvae occurred in a few farm houses and showed it was applicable to discriminate the virus disease from some bacterial ones.

**Kodama R and Nakasuji Y (1969). Bacteria isolated from silkworm larvae. II. Taxonomical studies on two strains of bacteria, E-5 and E-15, which showed pathogenic effects on aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 38:84-90.**

Investigations were made on the taxonomy of the gram positive cocci, strain E-5 and the gram negative rods, strain E-15, both of which showed pathogenic effects on silkworm larvae reared aseptically on an artificial diet.

The strain E-5 was found to share physiological characters between two species of the genus *Streptococcus*, *Str. faecalis* and *Str. faecium*, while the strain E-15 was found to be closely related to *Serr. picatorum* taxonomically.

*Str. faecalis-Str. faecium intermediate* E-5 was homofermentative lactic acid bacteria and required calcium pantothenate, nicotinic acid, biotin, folic acid, B6 group vitamin, arginine, glutaminc acid, glycine, histidine, isoleucine, leucin, methionine, threonine, tryptophan and valine essentially and alanine, cysteine, purines and pryrimidines stimulatorily for the growth.

**Iizuka T and Takizawa Y (1969). The aerobic bacterial flora in the gut of larvae of the silkworm, *Bombyx mori* L. II. The bacterial flora of the larvae reared on the artificial diet. *J. Sericult. Sci. Jpn.* 38:95-102.**

The aerobic bacterial flora in the midgut of silkworm larvae, *Bombyx mori* L., which were reared on two kinds of artificial diets, the one containing 50% mulberry leaf powder and the other without leaf powder was determined by means of three kinds of selective media. In both groups of larvae, fed either kinds of diet, the viable cells identified as *Streptococcus* sp. abnormally multiplied in the midgut from 3$^{rd}$ to 7$^{th}$ day of 5$^{th}$ instar larvae, eventually to $10^{10}$/ml

per larva. With the exception of the multiplication of *Streptococcus* sp., the bacterial flora in the midgut was similar to the previous observation (TAKIZAWA and IIZUKA, 1968), and *Staphylococcus aureus* was also a predominant strain. There was a large difference between the number of bacterial cells counted microscopically on smear samples and that of the viable cells counted by using three kinds of selective media. The combination method of PEA medium with HIA medium seemed to be the most effective for isolation of viable cells in the midgut, especially with respect to the growth of *Streptococcus* sp.

Bacterial strains were isolated from the fluid including digestive diets and two strains were identified as *Streptococcus* sp. 673 AD-4 and *Streptococcus* sp. 673 AD-5, which seemed to be *Streptococcus faecalis*. Therefore, AD-4 and AD-5 strains were added to new diets to determine the multiplication of these strains. In the diet containing 50% mulberry leaf powder, the multiplication of these strains was not recognized with 12 h, whereas, on synthetic diet, bacterial cells multiplied to $10^8$ (the first numbers 2 x $10^6$). In this flora, yeast was always isolated from the fluid including digestive diets. Some of the strains which were isolated were identified as *Saccharomyces* sp. 673 AD-1 and *Saccharomyces* sp. 673 AD-3.

**Kodama R and Nakasuji Y (1969). Bacteria isolated from silkworm larvae. III. Pathogenicity of lactic *Streptococci* and *Serratia piscatorum* for aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 38:103-109.**

Further studies were made on pathogenic effects of *Str. faecalis.-Str. faecium intermediate* E-5, *Serratia piscatorum* E-15 and various species of lactic acid bacteria on healthy silkworm larvae in 5$^{th}$ instar, which were reared aseptically on an artificial diet. The results obtained were:

In each case of *Str. faecalis-Str. faecium intermediate* E-5 and *Serr. piscatorum* E-15, the death of larvae was earlier with increasing amounts of living cells inoculated by feeding. However, a marked difference was observed between the number of living cells of these two species required for accomplishment of 100% mortality. *Str. faecalis-Str. faecium intermediate* E-5 grew rapidly in the gut of larvae after inoculation by feeding for four hourse, the diet being withheld from the larvae thereafter, but *Serr. picatorum* E-15 did not. Among lactic acid bacteria tested, only two strains of the genus *Streptococcus* roduced pathogenic effects on larvae, those of the genera *Pediococcus, Leuconostoc* and *Lactobacillus* did not.

**Yamaguchi K, Iwashita Y and Inoue K (1969). On themidgut-nuclear polyhedrosis in the silkworm, *Bombyx mori* L. III. Effects of high temperature treatment on the shape of polyhedron of the infected larvae. *J. Sericult. Sci. Jpn.* 38:157-162. [Japanese]**

**Kanke E and Iwashita Y (1969). Multiplication of the nuclear polyhedrosis virus in the midgut epithelium of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 38:163-167.**

The multiplication and development of the nuclear polyhedrosis virus in the nuclei of the midgut epithelium cells of the silkworm larva was studied by the light and electron microscopic observations. The differentiating forms as well as mature forms of the nuclear polyhedrosis virus were observed in the nuclei of the cylindrical cells of midgut epithelium. Single virus particles were developmental membrane in all cases. Accompanying the multiplication of virus, the infected nuclei of the cylindrical cells swelled and disintegrated. Polyhedra were rarely formed in the infected nuclei of the cylindrical cells, but in some cases, the development of minute polyhedra, less than one micron in diameter was detectable. It might be speculated that the inferior formation of polyhedron in the infected nucleus of the midgut epithelium might be due to the absence of basic substance or some enzymes necessary for the synthesis of nuclear polyhedron protein.

**Kurubayashi S and Higuchi T (1969). An investigation on silkworms presumed to have acariasis in silkworm farms. *J. Sericult. Sci. Jpn.* 38:168-175.**

The authors investigated silkworms presumed to have acariasis in 62 arms in the district of Maebashi and its surroundings of Gumma prefecture. Of a total of 62 cases investigated, 14 were established as damage caused by agricultural chemicals or flacherie or *Aspergillus* diseases, while in the other 48 cases, the cause of damage was not determined. Although the symptoms of the affected silkworms closely resembled acariasis reported by AMARI (1917) and others to be caused by *P. ventricosus*, we did not find *P. ventricosus* or any other mites injurious to the silkworm either on the body of the silkworm or in their rearing site.

On silkworm larvae that were discarded to a compost heap, we found two mite species *Macrocheles muscaidomesticae* and *Uropoda nipponica*, previously unknown to be injurious to silkworms. It is of interest to note that both species were located only between the maxilla and the first thoracic legs of the silkworm larvae. During this investigation, 7 kinds of mite species were found in the silkworm rearing site. Subsequent laboratory studies proved these to be harmless to the silkworm. In no case did we fid *P. ventricosus*, generally regarded as the most injurious parasite of all the known infecting mites. These results indicate that, most of the damage generally regarded in recent years as acariasis were caused by some unknown origin other than mite, and certainly non by *P. ventricosus*.

**Miyajima S and Kawase S (1969). Different infection response of silkworm larvae to the cytoplasmic-polyhedrosis virus with special reference to the locality of virus infection. I. Larval stage. *J. Sericult. Sci. Jpn.* 38:237-241.**

High sensitivity of the $5^{th}$ instar larvae to virus disease was observed when cytoplasmic polyhedrosis virus was injected into the posterior part (intersegmentally between the $8^{th}$ and $9^{th}$ segments) as compared to the anterior part (intersegmentally between the $5^{th}$ and $6^{th}$ segments). The dosage-infection response was always about 5,000 to 10,00,000 times higher in the posterior injection than in the anterior. The difference in the dosage infection response between posterior and anterior parts became larger with the advance of larval instar.

**Kuroiwa K, Miyashita T and Arai G (1969). Effects of some disinfectants upon the newly hatched larvae of silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 38:359-362. [Japanese]**

**Kobara R and Watanabe H (1969). Agarosegel electrophoresis of haemolymph proteins in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 38:386-394.**

Larval haemolymph in 80 strains of the silkworm was electrophoressed on agarose gel and 4 types of protein patterns were detected for the difference in number and mobility of main protein bands. Individual segregation of electrophoretic protein patterns in F1, F2 and back crossed generation indicated that the inheritance of each main protein band is controlled by codominant allelic genes. Autoradiograph of electrophoretic patterns of haemolymph proteins from larvae treated with carbon-14 labelled amino acids revealed that the incorporation of methionine into haemolymph proteins in the female, especially into the female associated proteins was much larger than that in the male. Furthermore, the autoradiography could detect the presence of over some protein bands into which tyrosine was well incorporated and yet the labeled protein turned rapidly.

**Kodama R and Nakasuji Y (1969). Bacteria isolated from silkworm larvae. IV. A study on the pathogenic mechanism of bacterial diseases in aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 38:406-412.**

Further studies were carried out on the pathogenicity of bacteria for aseptically reared silkworm larvae by the use of two species of pathogenic bacteria, *S. faecalis-S. faecium intermediate* E-5 and *Serratia piscatorum* E-15. The results obtained were:

*S. faecalis-S. faecium intermediate* E-5 grew rapidly in the gut of larvae, regardless of the coexistence with *Serratia piscatorum* E-15 and was recovered from the blood within 48 h after feeding the organism, but could not grow rapidly in the hemocoele. Accompanied by the growth of *S. faecalis-S. faecium intermediate* E-5 in the gut, lactate and acetate in the gut contents increased gradually, resulting in lowering of the pH values of the gut contents. *Serratia piscatorum* E-15 could not grow readily in the gut of larvae both starved and fed. But when this strain was coexisting with *S. faecalis-S. faecium intermediate* E-5 in the gut, the former could multiply in the gut somewhat later than the maximum growth of the latter. The pathogenic mechanism of bacterial diseases in aseptically reared silkworm larvae was discussed. The mechanism was postulated as follows: *S. faecalis-S. faecium intermediate* E-5 was associated with the bacterial disease primary invader, producing intestinal disease of chronic character and also playing an inductive role for the secondary invader, while *Serratia piscatorum* E-15 was associated with bacterial diseases as the secondary invader producing a lethal septicemia by the rapid growth in the hemocoele.

**Mukai JI, Takeya R, Inamasu M and Akune S (1969). A red fluorescent protein from silkworm digestive juice – a photosensitizing action on tryptophan and its biogenesis. *J. Sericult. Sci. Jpn.* 38:437-442.**

A blue coloured red-flourescent protein, previously isolated from the digestive juice of silkworm larvae in this laboratory was now shown to have a potent photosensitizing action in the visible light dependent destruction of L-tryptophan. The protein was inactive towards histidine and nucleic acid bases under the identical conditions. These results were discussed in relation to the mechanism of virus inactivating and rice plant withering actions of this protein.

**Nakasuji Y and Kodama R (1969). Bacteria isolated from silkworm larvae. V. Identification of gram-negative bacteria and their pathogenic effects of aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 38:471-480.**

Taxonomical and pathological studies were carried out on 26 strains of gram-negative bacteria isolated from epizootics in population of silkworms in various parts of Japan. The results obtained were:

These strains were classified into ten species: one strain of *Proteus vulgaris* HAUSER, one strain of *Proteus morganii* (WINSLOW *et.al.)* RAUSS, two strains of *Proteus inconstans* (ORNSTEIN) SHAT *et* CLARKE, five strains of *Serratia piscatorum* (LEHMANN *et* NEUMANN) BREED, two strains of *Serratia marcescens* BIZIO (nonchromogenic), three strains of *Aerobacter aerogens* (KRUSE) BEIJERINCK, six strains of *Aerobacter cloacae* (JORDON) BERGEY *et al.*, one strain related to *Alcaligenes bookeri* (FORD) BERGEY *et.al.*, one strain related to *Achromobacter superficialis* (JORDAN) BERGEY *et.al.*, and four strains related to *Pseudomonas ovalis* CHESTER. The pathogenic effects of these strains on healthy silkworm larvae in fifth instar, which were reared aseptically on an artificial diet were examined. The results obtained were:

*Serratia piscatorum, Serratia marcescens* (nonchromogenic), *Proteus vulgaris, Proteus morganii* and *Proteus inconstans* exhibited pathogenic effects on larvae either by feeding or injection, especially showing always 100% lethality by injection. One of three strains of *Aerobacter aerogenes* and five of six strains *Aerobacter cloacae* exhibited pathogenic effects on larvae only by feeding and *Achromobacter superficialis* related strain only by injection.

*Pseudomonas ovalis* related strains and *Alcaligenes bookeri* related strain exhibited little or no athogenic effects.

**Kobayashi M, Yamaguchi S and Yokoyam Y (1969). Influence of the larval development on the susceptibility of the silkworm *Bombyx mori* L. to the nuclear-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 38:481-487.**

A quantitative study was carried out to make clear the changes of response of the silkworm larvae, both resistant and susceptible strains, to the *per oral* infection of nuclear polyhedrosis virus during the 1st, 4th and 5th instars. Furthermore, the comparisons of response of the larvae to the inoculation of the virus particles by means of ingestion and subcutaneous injection were made during the 5th instar. The results were summarized as follows:

The susceptibility to the virus infection was high in newly hatched or newly moulted larvae and then became lower upto the moulting or mounting. However, this decrease line in susceptibility was interrupted by the temporary increase at the time of 24 h or 48 h during the three instars. On the other hand, in the case of the 5th instar, another increased point was observed at the time of 72 h or 96 h. especially pre-matured larvae of resistant strain was most resistant. The susceptibility decreased remarkably as the larvae grew older, and it was more significant in resistant strain than in susceptible strain. The difference in susceptibility with the progress of the larval development was that the subdermal infection is not so large as the *per oral* infection. From the results described above, it may be considered that the changes of susceptibility to the virus with the progress of the larval development are related to the inhibitory mechanism in the intestinal function.

**Kawakami K, Tobiyama N, Shimizu J, Yamada T, Yamazaki H, Ozawa A, Kataoka H and Muroga N (1969). Feeding of mulberry leaves polluted by the wing scales of the silkworm moth and growth condition of the larvae. *J. Sericult. Sci. Jpn.* 38:488-492.**

When silkworms are fed with mulberry leaves taken from a mulberry field adjacent to the egg production plant, they grow normally and very healthy in the spring season, while most of them usually suffer from the infectious flacherie (F) disease in the early and late autumn. This fact indicates that in the latter two seasons, mulberry leaves are attached with the moth scales polluted by the infectious flacherie germs as the egg production begins and a lot of the scales are sent with the wind to mulberry field, consequently the larvae eat the polluted leaves. Such a tendency is confirmed by exchange of the larvae or of mulberry leaves between the egg producing place and non producing place. If we rear young silkworm larvae with the leaves polluted by the scales, the majority of them die of flacherie disease even though they are reared with non polluted leaves in the latter stage. Meanwhile, when young silkworm larvae are fed with non polluted leaves, the disease hardly occurs even if they eat the polluted leaves in the older stage. This might be due to the difference in the resistance for the flacherie germ between growth stages.

## Vol. 39 1970

**Kobayashi M and Yamaguchi S (1970). Effects of inokosterone and ecdysterone on the susceptibility of the silkworm, *Bombyx mori* L., to a nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 39:33-36.**

In order to analyse the causal factors of the occurrence of nuclear polyhedrosis in the silkworm, the effects of mounting hormone on the host susceptibility to the virus were investigated with both the untreated and the ligated larvae of the 5th instar. Test 1: The larvae just after the 4th ecdysis were perorally inoculated with the virus and then Inokosterone or Ecdysterone was injected into them. Test 2: The larvae just before the mounting were inoculated

with the virus and were ligated behind the second abdominal segment at the time of 25 hours after the beginning of their spinning, to keep out the hormonal control inducing a larval metamorphosis. Inokosterone or Ecdysterone was injected into them immediately after the ligation. The results obtained are:

In test 1, any significant difference in the infectivity was not observed between the divisions injected with the moulting hormone and with the aseptic water. In test 2, on the other hand, the hormone injected significantly enhanced the susceptibility of the ligated larvae to the virus. From these results, it seemed reasonable to assume that the viral infection may be affected by the endocrine substance controlling the development of the silkworm larvae.

**Saijo S (1970). Studies on the chemical resistance of the parasitic fungi of *Aspergillus* disease in the silkworm, *Bombyx mori* L. I. Development of resistance to formaldehyde in the *Aspergillus*. *J. Sericult. Sci. Jpn.* 39:43-50.**

The author has studied on the development of the resistance of the silkworm to the disinfectant of the *Aspergilli* and selected resistant strains by the successive transfer increasing contact method. *Aspergillus* showed development of resistance to formaledehyde when treated with it due to a change in the morphological and physiological properties, and pathogenicity to silkworm. The change of resistance varied directly with the concentration of formaldehyde. The development of the pathogenicity was directly related to the physiological properties of the fungi, *ie*, its capacity to produce kojic-acid and pigments, capacity to decompose protein. Low concentration of formaldehyde reduced the capacity whereas high concentration of it increased that of the fungi. The remarkable variation observed in resistance to formaldehyde of *Aspergillus* of several strains was directly related to the ornamentation of its conidia and its wall.

**Nakasuji Y and Kodama R (1970). Bacteria isolated from silkworm larvae. VI. Relationship between the species of the genera *Micrococcus* and *Styphylococcus* and their pathogenicity for aseptically reared silkworm larvae. *J. Sericult. Sci. Jpn.* 39:187-193.**

A study was carried out on the relationship between the species of the genera *Micrococcus* and *Staphylococcus* and their pathogenicity for healthy silkworm larvae in 5$^{th}$ instar, which were reared aseptically on an artificial diet. 24 strains of both genera were used for the study. They consist of 14 strains isolated from epizootics in population of silkworm in various parts of Japan and also of ten strains maintained in the culture collection in our institute.

14 isolated were found to be closely related to the following species taxonomically. One to *M. freudenreichii*, one to *M. flavus*, four to *M. candidus*, five to *M. caseolyticus* and three to *S. epidermis*. The pathogenicity of these isolates and the strains maintained in our culture collection for aseptically reared silkworm larvae were tested by feeding the organisms to larvae and also by injuection into the hemocoele of larvae. The results obtained indicate that one of two strains of *M. flavus* and two of four strains of *M. candidus* were pathogenic only by injection. *M. freudenreichii, M. caseolyticus, M. aurantiacus, M. roseus, M. luteus, M. varians, M. rubens, M. afermentans, S. aureus* and *S. epidermidis* exhibited no pathogenicity either by feeding or by injection.

**Ohshima K (1970). *Nosema* disease of the dead silkworm, *Bombyx mori* after repeness. *J. Sericult. Sci. Jpn.* 39:243-247. [Japanese]**

**Iizuka T, Horie Y and Takizawa Y (1970). The aeobacterial flora in the gut of larvae of the silkworm, *Bomyx mori* L. III. Effect of dietary antibiotics on the multiplication of bacteria in the gut and on larval mortality caused by the rearing on artificial diets. *J. Sericult. Sci. Jpn.* 39:253-260.**

In the previous paper it has been reported that *Streptococcus* sp. predominantly multiplied in the gut of silkworm larvae fed on the artificial diet (IIZUKA and TAKIZAWA, 1969). The present study was undertaken to determine quantitatively the effect of various kinds of antibiotics on the bacterial flora including *Streptococcus* sp. in the gut of the silkworm fed on artificial diets and on the mortality of the larvae.

Two kinds of diets were used, one which contained mulberry leaf powder (diet A) and the other which did not (diet B). *Streptococcus* sp. began to multiply in the gut of the larvae reared on the diet B in the early stage of the $5^{th}$ instar and the number of living cells reached over $10^9$ per ml, resulting in high larval mortality. When the larvae were reared on the diet A, the multiplication of *Streptococcus* sp. was less, as compared with the larvae fed on the diet B and a maximal level of living cells was kept less than $10^7$ per ml. Such a difference in the multiplication of *Streptococcus* between two diets was also found by administering *Streptococcus faecalis* AD-4 to the larvae, although the lag phase for the multiplication was much shorter on both diets than those obtained without administration. Small numbers of living cells of bacterial species belonged to *Staphylococcus, Bacillus, Sarcina* and *Achromobacter* were isolated from the larvae. The addition of several kinds of antibiotics, especially tetracycline and spiramycin to the diet prevented effectively the multiplication of *Streptococcus* sp. and larval mortality was conspicuously lowered. The minimal effective doses of those antibiotics seemed to be 1,000 units/g of dry diet for penicillin, 10 mg/g for streptomycin, 1 mg/g for tetracycline, and 1 mg/g for spiramycin, respectively. Among those antibiotics, 10mg/g of streptomycin was found to be toxic for the larvae.

**Aruga H and Watanabe H (1970). Interference between UV-inactivated and active cytoplasmic-polyhedrosis viruses in the silkworm, *Bombyx mori* L. 1. A few facts controlling interference. *J. Sericult. Sci. Jpn.* 39:273-276.**

Studies have been carried out on the interference between UV inactivated hexagonal polyhedron virus and active tetragonal polyhedron virus in the cytoplasmic polyhedrosis of the silkworm. The results are as follows:

The degree of interference between the UV inactivated virus and challenge virus depended on the concentration of inactivated virus. When it was high, the interference was clearly observable and the incidence of cytoplasmic polyhedrosis by the challenge virus was less than control.

The degree of interference between UV inactivated virus and challenge virus was modified by the length of time of ultraviolet irradiation, the degree of interference being low when the treatment by ultraviolet irradiation was strong. The inactivated HC virus irradiated for 2 h showed higher capacity of interference than that irradiated for 3 h. It may be thought that the interfering capacity of the inactivated cytoplasmic polyhedrosis virus which suppresses the multiplication of challenge virus is, therefore, progressively destroyed by increasing the time of ultra-violet irradiation.

The degree of interference between inactivated virus and challenge virus was also modified by the difference of the dosage of challenge virus. When the dosage of challenge virus was markedly high and the incidence of cytoplasmic polyhedrosis was very high, the interference was not so clearly observable when compared with the case of low concentration of challenge virus.

**Aoki J and Yanase K (1970). Studies on the nutrition and metabolism of pathogenic fungi of Muscardine. (2) The influence of Amino Acid for the formation and Multiplication of Hyphal Bodies of *Spicaria fumoso-rosea* (Wize) Vassil. and of *Beauveria bassiana* (Bals.) Vuill. *J. Sericult. Sci. Jpn.* 39:285-292. [English]**

The availability of 17 aminoacids was investigated for the formation and multiplication of hyphal bodies of *Spicaria fumosa-rosea* and *Beauveria bassiana* in shake culture. In the productive process of hyphal bodies, different sorts of amino acid gave different influence upon the size and the rate of multiplication of the hyphal bodies. Tested amino acids were divided into three groups concerning their influence on the size of hyphal bodies, ie., hyphal bodies with long, moderate and short length. Furthermore, the difference of yield of hyphal bodies were observed in each group. In general, monoaminomonocarboxylic amino acids were superior for the formation of multiplication of hyphal bodies in both fungi. On the contrary, mono-aminomonocarboxylic and aromatic aminoacids were the least suitable. Various aminoacids, even in the same metabolic group, eg, glutamic acid, aspartic acid, aromatic acid, and pyruvic acid group gave varying effects. It was considered that the mode of availability of amino acid may have some correlation with metabolic pathway of amino acid.

**Yamaguchi K and Ayuzawa C (1970). Studies on the midgut nuclear-polyhedrosis of the silkworm, *Bombyx mori* L. (IV) Two previously undescribed virus strains, B, and $C_1$. *J. Sericult. Sci. Jpn.* 39:342-350.**

The authors discovered the two new virus strains of the midgut nuclear polyhedrosis of the silkworm, *Bombyx mori that* could be distinguished from the original virus strain by the shape of its polyhedra or inclusion bodies. These two strains are designated as strain B and $C_1$ respectively and the original virus strain is called strain A. The descriptions and characteristics of the diseases are presented below:

Strain B: Histopathological studies showed that a large number of rods were formed in the nuclei of cylindrical cells of infected midgut in the early stage of the diseases, with the advancement of infection, many polyhedra were formed in both nucleus and cytoplasm of the same cell. Polyhedron is seen as tetragon having round corners.

Strain $C_1$: A lot of the minute inclusion bodies were formed in the nuclei of infected midgut cylindrical cells, then the nucleus was hypertrophied and filled with inclusions.

Polyhedra of the strain B and the inclusion bodies of the strain $C_1$ were stained with bromophenol blue, orange G, azo-carmine and Heidenhain's iron alum hematoxylin after treating with 1 N HCl at 60°C.

**Yamaguchi K (1970). Studies on themidgut nuclear-polyhedrosis of the silkworm, *Bombyx mori* L. (V) A previously undescribed virus strain C2. *J. Sericult. Sci. Jpn.* 39:363-370.**

A new type of the midgut nuclear polyhedrosis was found in which minute inclusion bodies were contained within the nucleus and cytoplasm of the cylindrical cell of midgut of the silkworm. The size of the inclusion bodies depend on the rearing temperature after the inoculation, ie, the lower the rearing temperature, the larger the inclusions. They were amorphous grain less than 1 micron in diameter when the infected larvae were reared at 25°C and 30°c but appeared spherical in shape at 20°c. The spherical inclusions in the nucleus of cylindrical cell were smaller than those which were 10 microns in diameter in the cytoplasm. The spherical inclusion bodies were transformed into grain when the diseased larvae were reared at 25°C. The new virus strain is designated in this paper as strain $C_2$.

**Nakasuji Y, Kobayashi A and Kodama R (1970). Bacteria isolated from silkworm larvae. VII. Two patterns of the pathogenic effect exhibited by the strains belonging to the genus *Streptococcus*. *J. Sericult. Sci. Jpn.* 39:377-381.**

Further investigation was carried out on the pathogenicity of 22 strains belonging to the genus *Streptococcus* isolated from epizootics in population of silkworm in various parts of Japan, employing healthy silkworm larvae in the $5^{th}$ instar, which were reared aseptically on an artificial

diet. The pathogenicity of these strains was tested by feeding the organism to the larvae, and by injection into hemocoele of the larvae. The results obtained are:

It was found that there were two patterns – an intestinal disease and a septicemic disease – in the pathogenic effects exhibited by these strains except two non-pathogenic isolates.

The strains producing an intestinal disease exhibited the pathogenicity only by feeding. They were capable of growing in media of relatively high pH values, compared with the strains producing a septicemic disease and could not liquefy gelatin.

On the other hand, the strains producing a septicemic disease exhibited little or no pathogenicity by feeding, but exhibited a great pathogenicity by injection. They could liquefy gelatin and the maximum pH values for their growth were relatively lower than those of the strain producing an intestinal disease.

**Aruga H and Watanabe H (1970). Interference between UV-inactivated and active cytoplasmic-polyhedrosis viruses in the silkworm, *Bombyx mori* L. II. Silkworm strain and time interval inoculation. *J. Sericult. Sci. Jpn.* 39:382-386.**

Studies on the interference between UV inactivated and active virus of the cytoplasmic polyhedrosis have been carried out using F1 hybrids between Japanese univoltine and Chinese bivoltine strains of the silkworm, *Bombyx mori* in summer, early autumn and late autumn rearing seasons. The hexagonal polyhedron virus which had been inactivated by ultraviolet irradiation interfered with the tetragonal polyhedron virus and protected larvae against death due to the challenge virus. However, flacherie diseased larvae occurred by the feeding of inactivated virus suspended in distilled water. Consequently, the percentage of healthy larvae was not higher than control.

In the next set of experiments hexagonal polyhedron virus inactivated by UV irradiation was administered at the second instar larval stage and tetragonal polyhedron virus was inoculated at the $4^{th}$ or $5^{th}$ instar larval stage. The protection from the pathological consequences of the challenge virus was remarkable as in the case of 3 or 4 days interval reported in the previous reports.

It seems very likely from the above results that, the capacity of the inactivated virus to interfere remained unimpaired from the first to $4^{th}$ instar or from the $2^{nd}$ to the $5^{th}$ instar larval stages and the interference may be established even when the interval between the application of the inactivated virus and challenge virus is considerably long.

**Aruga H and Watanabe H (1970). Interference between cytoplasmic polyhedrosis virus and nuclear-polyhedrosis virus in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 39:420-424.**

Studies on the interference between interfering CPV forming tetragonal polyhedron in outline and the challenge NPV and the UV inactivated CPV forming hexagonal or tetragonal polyhedron and the challenge NPV in the silkworm have been carried out. When there was an interval of one day between the application of interfering virus and that of the challenge virus, two cases were observed, one was no effect of interfering virus on the incidence of nuclear polyhedrosis by challenge virus, the other was the reduction of nuclear polyhedrosis by the application CPV. When the tetragonal polyhedra of cytoplasmic polyhedrosis virus was administered to the $3^{rd}$ instar larvae, the incidence of nuclear polyhedrosis was lower than control.

When UV inactivated hexagonal polyhedra (HC) or tetragonal polyhedra (TC) of cytoplasmic polyhedrosis were fed to $2^{nd}$ or $3^{rd}$ instar larvae and challenge nuclear polyhedrosis

virus was administered to third or fourth instar larvae, the incidence of nuclear polyhedrosis by challenge virus was lower than the control. It seems likely that CPV and its UV inactivated virus interfere with nuclear polyhedrosis virus. But in the present experiment the diagnosis of nuclear polyhedrosis has been done by the observation of polyhedra in the nucleus of tracheal epithelium distributed to midgut, and has not on other tissues and organs. Consequently, further study is necessary to conclude that CPV and its inactivated virus interfere with NPV in the silkworm.

**Kodama R, Nakasuji Y and Nishio M (1970). Bacteria isolated from silkworm larvae. VIII. Experiments to protect aseptically reared silkworm larvae from bacterial diseases by oral administration of antibiotics. *J. Sericult. Sci. Jpn.* 39:425-428.**

Experiments were carried out to protect larvae from bacterial disease by oral administration of antibiotics, employing healthy silkworm larvae in the 5$^{th}$ instar, which were reared aseptically on an artificial diet. The oral administration of the antibiotics, which suppressed effectively the growth of the pathogenic bacteria used in the test tubes, made it possible to protect the larvae from diseases to be caused by the bacteria orally inoculated. However, there were marked differences between the amounts of antibiotics in the test tubes required for suppressing the growth of the bacteria and those in the diet required for protecting the larvae from bacterial diseases.

**Aoki J (1970). Hyphal bodies of the insect pathogenic fungi: The reconsideration on the Japanese name for hyphal bodies. *J. Sericult. Sci. Jpn.* 39:458-461. [Japanese]**

## Vol. 40 1971

**Kodama R and Nakasuji Y (1971). Bacteria isolated from silkworm larvae. IX. The behaviour of antibiotics added to an artificial diet. *J. Sericult. Sci. Jpn.* 40:8-12.**

The behaviour of antibiotics were investigated, when they were added to an artificial diet and then taken in the gut of larvae with the diet. Some of the antibiotics tested were inactivated by keeping them in contact with the digestive juice of larvae. Chloramphenicol and triacetyloleandomycin were stable, while tertiomycin and spiramycin were relatively unstable. Using chloramphenicol, following results were obtained. When the antibiotic was added to the diet, it was considerably adsorbed on the diet itself. At the period of roughly 4 h after the larvae began to ingest the antibiotic-containing diet, the amounts of the antibiotic in the gut contents reached maximum, and in the vicinity of the same period of time the amounts of the antibiotic excreted in the feces began to increase. On the contrary, when the antibiotic containing diet, which was continued to be ginven till that time, was replaced by the antibiotic omitted diet, the antibiotic could not be detected from the gut contents at the period of 10 to 11 h after the replacement.

**Miyajima S (1971). The inhibitory effect of 5-fluorouracil on the outbreak of infectious flacherie in the silkworm, *Bombyx mori*. (IV) On some administration method of 5-fluorouracil. *J. Sericult. Sci. Jpn.* 40:18-22.**

In the inhibitory effect of 5-fluorouracil on the outbreak of infectious flacherie, the shorter the time after inoculation the less the incidence of infectious flacherie occurred, especially in case of administration within 12 h after virus inoculation. 5-flourouracil has not virudicidal activity *in vitro* during 24 h at 4°c or 3 h at 37°C in mixture. In the physiological effect of 5-flourouracil on egg production of female, hatchability of eggs oviposited by the treated silkworm was not different significantly among the treated series. Sterilizing effect of 5-flourouracil on the silkworm was unable to be found under the concentration of 5 x $10^{-4}$ M in each treatment.

**Kurata K (1971). On symptoms of silkworm, *Bombyx mori*, infected with a cytoplasmic-polyhedrosis virus under aseptic condition. *J. Sericult. Sci. Jpn.* 40:32-36.**

The silkworm larvae inoculated with CPV in each larval stage were studied to make clear the mode of symptoms of the polyhedrosis under aseptic condition. The condition was brought by rearing them on the artificial diet in a test tube individually at 27-28°C. The results are as follows:

When larvae were inoculated with CPV in the 1st instar, the infected larvae died of the disease 7 to 53 days after the inoculation. About 80% of them were dead in the 5th and 6th instars. Some of the larvae infected with CPV became imagos and ovipositioned. The amount of eggs was about a quarter of those of normal imagos. In comparision with normal imagos, the infected ones were prolonged their larval stage more than 18 days. When older larvae inoculated, the number of imagos with polyhedra in their intestines increased. pentamoultours appeared in the tetramoulting race, when they were inoculated with CPV. The younger the larvae were inoculated, the more the pentamoulters appeared. The infected larvae and imagos retained polyhedra in their midguts throughout the remainder of their life since 4 days after administration, even when they were inoculated on the second day of the 1st instar. The 1st instar larvae were inoculated with CPV and their progeny was examined to elucide the virus transmission to following generations. As a result, no evidence of trans ovum transmission of virus was observed, even when the progeny was treated with low temperature (5°C, 24 h) or was fed sodium fluoride, on the first day of the 5th instar.

**Watanabe H and Aruga H (1971). The effect of molt on the development of nuclear polyhedrosis in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 40:37-41.**

Silkworm larvae, *Bombyx mori* at various stages of infection with a nuclear polyhedrosis virus were induced to moult by the injection of Ponasterone A, an Ecdysone analogue which had been found recently in plants. By means of autoradiography with $^{3}$H-thymidine and $^{3}$H-tryosine, the development of the disease in the larva during the moult was compared with that in the unmoulted larva.

In an infected cell, the incorporation of $^{3}$H-thymidine into the nucleus was most prevalent during the stages of chromatin condensation and ring zone formation. This situation was almost similar whether the infected larvae were moulting or not moulting. In an infected cell at the stage of polyhedron formation, $^{3}$H-tyrosine was incorporated mostly onto polyhedra in unmolted larva, but the incorporation of the label into polyhedra was greatly reduced in larva which had been induced to moult by injection of Ponasterone A. An infected hypodermal cell before the formation of the polyhedra was able to form new cuticle during the moult, and much $^{3}$H-tyrosine was incorporated into the newly developed cuticle. On the other hand, a hypodermal cell, which contained polyhedra, was unable to form new cuticle and the larva could not undergo ecdysis. These results indicated that, when the host larva was in moult, the viral DNA was synthesized, but the synthesis of polyhedron protein was reduced. The moulting larva in an advanced stage of infection could not undergo ecdysis because of the inability of the hypodermal cell to form a new cuticle.

**Sekijima Y (1971). Studies on serological diagnosis of the infectious flacherie in the silkworm, *Bombyx mori*. 1. Demonstration of specific antigen in extract from the silkworm larvae infected with flacherie virus by precipitation reaction. *J. Sericult. Sci. Jpn.* 40:49-55.**

The experiment was performed for the purpose of quick diagnosis of infectious flacherie of the silkworm. Rabbits were immunized with extract of silkworm larvae infected with flacherie virus. Anti flacherie virus infected silkworm extract immune sera were tested against normal and

flacherie virus infected silkworm extracts by the precipitation techniques of ring test and agar gel diffusion.

At least three precipitation bands were detected against flacherie virus infected silkworm extract. One of them was common to the extract from normal silkworm larvae. After absorption of the antiserum with the extract from normal larvae, at least two specific precipitation bands were detected not only with the purified extract, but also with the crude extract of larvae collected from rearing houses of farmers.

**Iizuka T (1971). The aerobic bacterial flora in the gut of larvae of the silkworm, *Bombyx mori* L. IV. On the multiplication of some enteric bacteria in the gut of silkworm larvae reared on the artificial diet including antiseptics under axenic condition. *J. Sericult. Sci. Jpn.* 40:86-90.**

Each of gten predominant enteric bacteria, previously isolated from the gut of larvae of the silkworm by Takizawa and Iizuka (1968) was allowed either to be inoculated onto the artificial diet or to be fed to the fifth instar larvae of the silkworm growing on the artificial diet axenically.

The multiplication of these ten enteric bacteria was hardly observed on the artificial diet including antiseptics used in the present study. Twenty four hours after the inoculation of *Achromobacter parvulus* or *Alcaligenes metalcaligenes* on to the diet, neither of them could beisolated from the diet, but *Streptococcus faecalis* and *Bacillus cereus* could be isolated for a period of 7 days. In this case the number of bacteria to be isolated was almost constant during this period.

When each species of bacteria was fed perorally to the larvae of the fifth instar, the pathogenicity was recognized with *Streptococcus faecalis,* but not with any of other nine bacterial species. With the exception of the multiplication of *Streptococococcus faecalis*, number of living cells of *Staphylococcus epidermidis, S. aureus* and *Bacillus cereus* were scarcely isolated after the first twenty four hours.

When the larvae were fed with *Staphylococcus epidermidis* in combination with any species of other gram-negative bacterial numbers of *staphylococcus epidermidis* isolated from the fifth instar larvae did not differ largely from these obtained in monoxenic culture.

**Watanabe H and Namura H (1971). Fecundity of adult of the silkworm, *Bombyx mori*, infected with a cytoplasmic-polyhedrosis virus in the pupal stage. *J. Sericult. Sci. Jpn.* 40:145-146.**

Silkworm pupa, *Bombyx mori* was susceptible to intrahemocoelic infection with a cytoplasmic polyhedrosis virus and the virus developed well in the midgut. However, infected pupa was quite immune to a lethal infection and developed into an adult with normal appearance. The fecundity of diseased female adult was not significantly different from that of the uninfected female. However, there was a little reduction in fertility when either one of the mating adults was infected.

**Murakoshi S, Sugiyama J and Ohtomo T (1971). Studies on the *Aspergilli* from silkworm, *Bombyx mori*. (I) The occurrence of aflatoxin-producing strains in *Aspergillus* strains isolated from Sericultural farms and their toxic effects on silkworm larvae. *J. Sericult. Sci. Jpn.* 40:167-175.**

209 strains of *Aspergilli* were isolated from dust samples collected at many Sericultural farms in Kanagawa Prefecture, Japan, of which 98 isolates were examined to clarify their taxonomic position and presence of any mycotoxin producing strains in the isolates. The test for

toxic effects of chloroform layer from fungal culture was made with the silkworm assy method newly proposed here, by the use of feeding trials added to artificial diets.

Of 98 isolates examined, 50 strains revealed a conspicuous toxic and probably pathogenic effects to silkworm larvae, and furthermore, most of them were identified as *Aspergillus flavus* Link ex Fr. When methanol extracts derived from the chloroform layer were chromatographed on thin layer chromatoplates and inspected in the Chromato-Viewer, the four aflatoxins were separated, $B_1$, $B_2$, $G_1$ and $G_2$. Also ultraviolet absorption absorption spectra of each fluorescent spt agreed with those previously obtained. The presence of aflatoxins and other fluorescent spots in 37 *A. flavus* isolates varied significantly with strains. In effects of each fluorescent spot to silkworm larvae, the spt $B_1$ was highly toxic, showing 100% mortality in 5 days after treatment in 10 silkworm larvae tested, while the spot $G_1$ was moderately toxic. Even in 10 silkworm larvae tested fed on artificial diets containing 1.5 ppm of pure aflatoxin $B_1$, highly toxic effects (attaining 100% mortality) were also observed after 6 days.

It seems that in *A. flavus* isolates there is a notable degree of correlation between the presence of aflatoxins and their pathogenicity to silkworm, their tolerance to formalin, and their ability to produce pigments.

**Aruga H and Watanabe H (1971). Interference between UV-inactivated and active cytoplasmic-polyhedrosis viruses in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 40:176-180.**

In this study, interference between the UV-inactivated hexagonal polyhedron virus and active tetragonal polyhedron virus in the cytoplasmic polyhedrosis of the silkworm was tested of the case where the inactivated virus was applied to the larva in a form of dust with several diluents. When the UV inactivated virus was fed to the larva with white kaoline or Japanese acid clay as diluent, the inactivated virus interfered the active virus, which was administered lately, and lowered the incidence of cytoplasmic polyhedrosis. However, the degree of interference in this case was much less than that in the case reported previously (Aruga and Watanabe, 1970) where the inactivated virus was applied in a form of water suspension. On the other hand, when the inactivated virus was applied with slaked lime, whitecarbon, or startch, interference of the inactivated virus to the active virus infection was not clearly observed. As these diluents showed effects of enhancing or suppressing the virus infection in the silkworm, this might lead to the uncertain interference.

**Nunome J, Shikata M, Murata T and Matsumoto T (1971). Studies on air hygiene of silkworms VI. Detection of organic gases generated from dead silkworms and resistivities of silkworms to those gases. *J. Sericult. Sci. Jpn.* 40:209-216.**

Studies were made of the generation of organic gases from dead silkworms and of the resistivities of $5^{th}$ instar silkworms to those gases except indol. Generation of indol gas, propionic acid gas, iso-valeric acid gas, n-butyric acid gas and perhaps iso-caproic acid gas from silkworms dead of infectious flacherie or nuclear polyhedrosis was confirmed as a result of gas chromatographic analysis. These gases increase as the decomposition of silkworm body advances. Resistivities of $5^{th}$ instar silkworm, both resistant and susceptible strains, to a fixed concentration of propionic acid gas, n-valeric acid gas and iso-valeric acid gas were investigated. 1. Degree of physiological disorder produced by propionic acid gas and iso-valeric acid gas were larger than that by n-valeric acid gas. 2. There was a tendency that infectious flacherie increases by propionic acid gas and nuclear polyhedrosis by iso-valeric acid gas. 3. Many infectious flacherie have been seen until gluttonous stage and after this stage they were complicated with nuclear polyhedrosis. 4. Resistivities of $5^{th}$ instar silkworm to those gases mentioned above differ with strains. 5. Many sick silkworms by the complication of infectious flacherie and nuclear polyhedrosis wee seen in Japanese race.

**Aruga H and Iwashita Y (1971). An effect of administration of UV-inactivated cytoplasmic-polyhedrosis virus on the incidence of flacherie caused by bacterial infection in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 40:217-220.**

In this study, an effect of administration of UV inactivated cytoplasmic polyhedrosis virus in the silkworm larvae on the incidence of bacterial flacherie was investigated. The larvae were first administered with the inactivated virus, treated subsequently with peroral inoculation of E5 strain of *Streptococcus faecalis* and E15 strain of *Serratia*, and then the incidence of flacherie caused by the bacterial infection was compared with that in the larvae treated with the bacteria alone. However, the results of the test indicated that administration of the UV inactivated virus to the larvae had no effect on the incidence of flacherie caused by the bacterial infection, even in the bacterial infection was enhanced by low or high temperature treatment.

**Miyajima S (1971). Susceptibility during the larval growth of silkworm to a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 40:227-230.**

The susceptibility to a cytoplasmic polyhedrosis virus during the larval growth of silkworm, *Bombyx mori* L., was investigated. Each larvae were injected with appointed volume and concentration of virus suspension into dorsal part (inter-segmental part between $8^{th}$ and $9^{th}$ segments). Results obtained are as follows:

Infectivity titer (-log $ED_{50}$) in case of inter-segmental inoculation of the virus in the $1^{st}$ day of $3^{rd}$ instar larva just after ecdysis was ≥ 7.8, but was much lower (4.1) in the $3^{rd}$ moulting larva. The same result was obtained between $1^{st}$ day of $4^{th}$ instar larva just after ecdysis and $4^{th}$ moulting larva. When each larva of $3^{rd}$, $4^{th}$ or $5^{th}$ instar just after ecdysis was injected with various concentrations of the virus suspension, the highest rate of infection was shown at the youngest stage among these three larval stages. Among various stages of $5^{th}$ instar larva injected with the virus, the highest infection response was revealed at the stage of larva just after ecdysis among other ones, and the susceptibility to the cytoplasmic polyhedrosis virus decreased gradually during the larval growth. From the results mentioned above, it is considered that the changes in response, *ie*, susceptibility of the silkworm larval growth to a cytoplasmic polyhedrosis virus might be affected by hormone, virus inhibitory factor or like substances, etc.

**Utsumi S (1971). Preparation of *Bacillus thuringiensis* as an insecticide contained no living cells. *J. Sericult. Sci. Jpn.* 40:269-274.**

In Japan, *Bacillus thuringiensis* and its relatives have not been used as an insecticide for the protection of silk industry which may be destroyed by the spraying of the living microbes. In this connection, the following treatments to the bacteria succeeded in preparation of an insecticide having no ability to multiply themselves but the serious toxicity to the lepidopterous insects.

The bacteria were treated with 0.5% hydrogen peroxide or 0.25% β-propiolactone for 24 h and then they were washed with distilled water by centrifuging. The precipitate contained the dead cells and crystal toxin was dried to brown powder under vacuum. The preparation showed high toxicity against *Dendrolimus spectabilis*, *Pieris rapae* and *Parnana guttata*. The larvae fed the preparation were died by the paralysis within 50 h. But sonication or adition of acetone to the bacterial suspension were insufficient to destroy the growth ability of bacteria. The treatments with formalin solution, bleach powder or hypochlorous acid were inadequate for the purpose. It is considered that the preparation can be used in practice as an insecticide without the bacterial contamination in Sericultural area.

**Watanabe H, Aruga H and Namura H (1971). An effect of molt on the development of cytoplasmic polyhedra in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 40:275-280.**

A study was made on the growth of cytoplasmic polyhedra in the silkworm, *Bombyx mori*, during moult. During the moulting stage of the infected larva, the development of polyhedra in number and size was almost inhibited, but the development was continued to proceed after the moult. When the silkworm was injected with Ponasterone A, an Ecdysone analogue, the larva was forced into a moult several hours after the injection. In such larva which was induced to moult, the development of polyhedra was also inhibited. Autoradiographic studies with $^{3}$H-tyrosine (protein precursor) and $^{3}$H-uridine (RNA precursor) revealed that in the molting larva infected with a cytoplasmic polyhedrosis virus, the synthesis of protein in the infected cells was greatly suppressed during the moult and synthesis of RNA was also on the marked decrease. Thus, the suppression of polyhedral growth during the molting stage of the infected larva might be mainly attributable to a great lack of polyhedral protein synthesized in the infected cells.

**Suto C and Kawase S (1971). Hemagglutination with flacherie virus of the silkworm. *J. Sericult. Sci. Jpn.* 40:288-292.**

Hemagglutination reactions with flacherie virus (FV) and the supernatant of FV infected midgut homogenate of the silkworm were examined using erythrocytes from various animals. The results obtained are as follows:

FV infected midgut homogenate (20%) in phosphate buffered saline was centrifuged at 10,000g for 30 min and the resultant supernatant was added to erythrocyte suspension of chicken, rat, sheep, goat, human or mouse. Among them, only mouse erythrocytes were agglutinated clearly with the supernatant. The most clear hemagglutination was obtained using the erythrocyte suspension (0.25~0.5%) in phosphate buffered saline at room temperature 22°C. The optimal pH range was 6.6 to 8.5 in phosphate buffered saline. The hemagglutination occurred in the sediment of 105,00g of FV infected midgut homogenate and purified viruses prepared from the sucrose density gradient centrifugation. The hemagglutination was inhibited by FV antiserum. From these results, it is highly probable that the hemagglutination of mouse erythrocyte is due to flacherie virus itself.

**Utsumi S and Kurisu K (1971). On the preparation of aseptic diet by addition of β-propiolactone for rearing of silkworm, *Bombyx mori. J. Sericult. Sci. Jpn.* 40:343-349.**

It has previously been shown by the present authors that β-propiolactone (PL) possesses the powerful disinfectant effect on the pathogenic microorganisms of silkworm diseases and that PL is readily hydrolyzed to form β-Rydroxypropionic acid as a substance with low toxicity. Thus, it was supposed that PL could be used as the disinfectant for the preparation of the aseptic diet for the silkworm.

To know the disinfectant effect on the microorganisms in the diet, each of 0, 0.025, 0.05, 0.10, 0.20, 0.40 or 0.80% PL was added to the diets respectively with combination of steaming at 100°c for 15 min. Then the number of live bacteria in the diet was counted by plate method. Subsequently, the efficiency of PL addition on the preparation of the aseptic diet, and the effect of PL on the growth of silkworm were investigated.

An aseptic diet was prepared successfully by combination of the addition of PL in concentration of 0.05% and steaming for 15 min at 100°C. Without steaming, PL was needed in concentration of 0.8%. The larvae fed on the diet prepared with PL in 0.075% and then steamed grew well and pupated like fed ones on the autoclaved diet. Those fed on the diet containing 0.8% PL, however, did not grow well and not reached the 3$^{rd}$ instar within 11 days. Thus it was considered that the steaming treatment was essential for preparation of diet.

From the results mentioned above, it may be suggested that the aseptic diet for silkworm is easily prepared by an appropriate combination of addition of the PL and steaming.

**Watanabe H (1971). Susceptibility to virus infection in the silkworm, *Bombyx mori*, applied topically with sublethal dosages of insecticides. *J. Sericult. Sci. Jpn.* 40:350-356.**

This study was conducted to know an effect of sublethal contamination of insecticides in the silkworm rearing on the susceptibility of the silkworm to virus infection. When sub-lethal dosages of DDT and Sumithion, an organophosphorus insecticide were applied topically to the silkworm larvae, there appeared no sign of toxication such as paralysis, vomiting, reduction of feeding and growth. However, larvae treated with Sumithion were more susceptible to *per oral* infection with a nuclear and a cytoplasmic polyhedrosis virus than larvae not treated with insecticides, whereas larvae treated with DDT showed an increased susceptibility to a nuclear polyhedrosis virus.

**Utsumi (1971). An inhibitory effect of the compounds obtained by the addition reaction of β-propiolactone with nucleic acid and related substances on the incidence of nuclear polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 40:375-386.**

The compounds resulted from addition reaction of β-propiolactone (PL) with guanylic acid (GMP) and its related substances were easily prepared. The prepared addition compound of PL with 5' GMP (5' GMP-PL) was studied on its chemical properties and on the effect on the incidence of nuclear polyhedrosis of silkworm.

All compounds obtained by addition reaction of PL with GMP and related substances showed fluorescence by ultraviolet irradiation. It was concluded that 5' GMP-PL consisted mainly of 7- (2-carboxyethyl)-5'-guanylic acid, but it was mixed with another minor component. The two kinds of components were separated by chromatography on Dowex 1 (formage) or on paper chromatography (P.C). The main component was changed into 7-(2-carboxyethyl) guanine by hydrolyzation with N-HCl at 100°C for one hour. But minor component was destroyed at the structure of nucleic acid base by the treatment, therefore hydrolysate of minor component did not show the UV absorption spectrum of nucleic acid. By the action of 5' nucleotidase on the main and minor component of 5' GMP-PL, two kinds of substances were obatained which coincided on PC with the two spots on PC of GR-PL.

PL addition compounds with guanylic acid and its related compounds were found to have an inhibitory effect on the incidence of nuclear polyhedrosis when given orally or by injection to the silkworm.

**Utsumi (1971). Studies on the inhibitory mechanism of β-propiolactone reacted guanylic acid on the incidence of nuclear polyhedrosis in the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 40:431-438.**

It has been shown by the author that β-propiolactone (PL) has a powerful reactivity with nucleic acid and proteins. The reacted substance of PL with 5' GMP (5' GMP-PL) was easily prepared as mainly consisting of 7- (2-carboxyethyl)-5'-guanylic acid and it has an inhibitory effect on the incidence of nuclear polyhedrosis in the silkworm. In this report the inhibitory mechanism was studied from the point of the nucleic acid metabolism.

5' GMP-PL has an obstructive effect on the phosphorylation of the nucleotides such as AMP and GMP in the reaction of the crude enzyme extracted from bakers' yeast. The incorporation of $^{32}P$ into RNA fraction was decreased, when the silkworm were administered with 5' GMP-PL. And the amount of RNA and DNA in the silkworm decreased by injecting 0.5 mg of 5' GMP-PL per g of body weight. The incorporation of 5' GMP-PL into RNA of that silkworm was observed and the RNA differed in the base ratio of nucleic acid compared with the control one, especially reduced ratio in the purine nucleotides. At various intervals during the progress of virus multiplication, the amounts of RNA and DNA were measured in the pupae inoculated with virus and their control ones both of which had been injected with 0.5 mg of 5'

GMP-PL per g of body weight. The synthesis of DNA and RNA were inhibited in the pupae injected with 5' GMP-PL.

From the result obtained, the inhibitory action of 5' GMP-PL on an incidence of the nuclear polyhedrosis was revealed by disturbing the synthesis of nucleic acid, suggesting that the genetic code in associated with viral multiplication may be disrupted.

**Kawase S (1971). Different infection response of silkworm larvae to the cytoplasmic-polyhedrosis virus with special reference to the locality of virus injection. II. Detection of the portion showing highest infection response to the virus. *J. Sericult. Sci. Jpn.* 40:459-462.**

The part showing the highest infection response of 5$^{th}$ instar silkworm larvae to cytoplasmic polyhedrosis virus injections was the limited area between 7$^{th}$ and 10$^{th}$ segments. No difference was observed between dorsal and ventral parts of the same segment on the infection response of the silkworm to the virus infection. When the virus was injected into the dorsal vessel of the silkworm larvae, the injection into posterior part (between 7$^{th}$ and 8$^{th}$ segments) of the dorsal vessel brought higher infection response than that of anterior part (between 3$^{rd}$ and 4$^{th}$ segment). From these results, it may be suggested that the invasion of the virus into midgut epithelium is most favourable in the midgut area between 7$^{th}$ and 10$^{th}$ segments.

## Vol. 41 1972

**Kobayashi M (1972). Penetration of polyhedrosis viruses into the cultured midgut cells of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 41:1-6.**

A series of experiments were conducted on the entry of the CPV and NPV in silkworm midgut epithelial cells cultured *in vitro*. The following stages of penetration of the CPV into a cell were postulated from the observations in electron microscope. The first stage of the penetration appears to the attachment of the viral projection to the surface of the cell. In the next stage, the virus particles are attached much more closely to the cell surface, and penetrate through the projection into the cell. The final stage, the beginning of which is evident within 10 min after the inoculation of the virus, is characterized by the release of core substance into the cell.

The core substance appeared to be released as a filament, and injected. This was also suggested by the presence of empty particles on the cell surface. Phagocytosis did not seem to play a part in CPV penetration. The projection through which the core substance entered the cell did not contract during the penetration stage. The inner shell or the core membrane appeared to play an important role in the transfer of core substance from virus to cell. On the entry of the NPV a definitive conclusion could not be obtained, but a few micrographs suggested the possibility that the fusion of viral envelop with the plasma membrane is the mode of NPV penetration into the gut cells.

**Kodama R and Nakasuji Y (1972). Bacteria isolated from silkworm larvae. X. Inhibition of development of viral diseases in gnotobiotic silkworm by nalidixic acid. *J. Sericult. Sci. Jpn.* 41:7-14.**

Employing gnotobiotic silkworm larvae reared on an artificial diet, chemical agents protecting the larvae from viral diseases were researched. Following results were obtained. By the oral administration of nalidixic acid (NA) the development of infectious flacherie and nuclear polyhedrosis to be caused by the viral pathogens orally inoculated was inhibited, but that of cytoplasmic polyhedrosis was not inhibited. When the infectious flacherie virus was used as the test pathogen, more NA was required for inhibiting the development of disease with increasing

concentration of viral suspension added to the diet. When very high concentration of the viral suspension were adopted for inoculation, the inhibition by NA was not achieved.

The development of disease under synergistic effect observed b/w the pathogenicity of the IFV and that of *S. faecalis, S. Faecium* intermediate G-27, one strain of pathogenic streptococci, was inhibited by the combined administration of leucomycin and NA. In this experiment the lethality considerably lowered by the oral administration of leucomycin alone, but did not so particularly by that of NA alone. Among NA and its related compound tested, though they were small in number, only NA exhibited satisfactorily the inhibitory effect on the development of viral disease. The good inhibition by NA was produced when this compound was administered both before and after the inoculation of the virus, and the inhibitory effect was greater with increasing period of time of the administration. The amount of NA required for inhibiting the development of disease decreased by addition of fructosazine to the diet.

**Miyajima S (1972). Effect of a high temperature on the incidence of cytoplasmic polyhedrosis of the silkworm, *Bombyx mori*. II. Period in a high temperature on the incidence and adsorption of the virus to the midgut cell. *J. Sericult. Sci. Jpn.* 41:61-68.**

When rearing temp was changed from 35°C to 25°C after virus inoculation, the longer the period in a high temp, the lower the incidence of cytoplasmic polyhedrosis. The similar results were obtained when rearing temp was changed from 25°C to 35°C.

In order to know the early initiation of virus multiplication, the adsorption ability of virus to the midgut cell at a diff temp was examined. Same quantities of the midgut samples from healthy sw were obtained and they were added to continuous diluted virus suspension for 3h at 25°C or 35°C. After centrifugation, the supernatant was injected into another silkworm larvae to estimate the virus titration. Hemagglutination test was also used. One hour after virus injection to the silkworm larvae, the haemolymph or midgut homogenate from them was injected into another silkworm larvae and the amount of virus was estimated with bioassay and with precipitin ring test. From these results, it was ascertained that there were no different adsorption abilities of midgut to the virus between 25°C and 35°C.

The extraction of the interferon like substance(s) was tried by the modified method of NAGANO *et.al*., (1966) from the silkworm larvae reared at a high temp, but no activity was recognized. From these results, it is assumed that the cause of suppression of the virus multiplication at a high temp is neither virus adsorption process to the midgut cell, nor the product of interferon like substances(s) in the silkworm. The problem in which stage the virus multiplication is suppressed at a high temp remains yet to be determined in the near future.

**Tsutsui R, Mukai J, Watanabe T and Akune S (1972). Toxic crystals produced by *Bacillus thuringiensis* T84 A1. (III) Column chromatography of the toxic protein. *J. Sericult. Sci. Jpn.* 41:170-174.**

In order to investigate the chemical structure of toxic protein produced by *Bt* T84 A1, the present investigation was carried out by column chromatography on CM-cellulose, DEAE-cellulose and gel filtration. The results obtained were as follows:

The toxic protein was excluded by Sephadex G-200, and was not adsorbed to CM-cellulose. After a linear gradient elution from DEAE-cellulose column the toxic protein was separated into several peaks, while the similar elution in the presence of 8 M urea resulted in an almost single elution peak. This suggests that the toxic protein undergoes reversible dissociation and association.

**Hukuhara T, Namura H and Kobayashi M (1972). Aberrant Polyhedra of the Cytoplasmic-Polyherosis Virus of the Silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 41:187-196. [English].**

Aberrant polyhedra were found in the midgut cells of larvae of the silkworm, *Bombyx mori* and of the fall webworm, *Hyphantria cunea*, which had been infected with the hexahedron strain of the cytoplasmic polyhedrosis virus of the silkworm. In the silkworm cells the aberrant polyhedra occurred very infrequently (1.6%), but in those of the fall web worm, they were present in considerable numbers (11.9%). The aberrant polyhedra appeared to be composed of two or three cubes, which had various relationships with one another. In some cases all or part of the faces, edges, etc., of one cube were parallel to the similar elements of a second cube. In other cases, the relation appeared to be irregular. Crack like spaces were commonly found within the abberant polyhedra at the boundary, where the crystalline masses of polyhedron protein molecules met.

**Kobayashi M and Yamaguchi S (1972). An effect of ecdysterone on the development of nuclear- polyherosis in the isolated larval abdomen of the Silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 41:275-278.**

Susceptibility of the isolated larval abdomen of the silkworm to a nuclear polyhedrosis virus was increased by the injection of ecdysterone. Autoradiograms showing incorporation of $^{3}$H-thymidine in the isolated larval abdomens revealed that the incorporation of $^{3}$H-thymidine into the nucleus of infected cell was enhanced when the host was received an injection of ecddysterone. These results indicated that a nuclear-polyhedrosis virus developed more rapidly in the ligated larva, which had been inoculated with ecdysterone and induced to pupate, than in the larva without ecdysterone.

**Inoue H (1972). Effects of physical and chemical treatments on the properties of infectious flacherie virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 41:279-284.**

The IFV was examined by the bioassay to newly-hatched larvae of the silkworm and the antigenisity of IFV was observed by a fluorescent antibody technique. The results were summarized as follows:

The infectivity of the purified IFV was reduced, when IFV was buffered at pH 2 but was unaffected at the pH range from 3 to 12. IFV retained the most stable antigenisity at pH 6. The purified IFV lost the infectivity on the treatment of temperature at 70°C for 20 min, but was not affected at 50°C for 60 min. The antigenisity of IFV was disappeared at 50°C for 20 min and immediately at 70°C, when IFV was left standing in heated-distilled water. On the other hand, the antigenisity of IFV was little affected at 50 and 70°C for 6 h when IFV left standing in an incubator under the dry condition. The purified IFV was inactivated by the irradiation of UV light (2537 Å) for 120 min at 40 cm from the light source. The effect of UV on the antigenisity of IFV was not observed in 6 h. The purified IFV showed resistance to pronase, trypsin and pepsin at 30°C for 24 h.

**Tanaka S (1972). Effect of a new microsporidiosis of the silkworm, *Bombyx mori*, on the cocoon weight, the cocoon shell weight and the number of eggs laid. *J. Sericult. Sci. Jpn.* 41:305-308.**

The purpose of this study was to clarify the effect of a new microsporidian infection to the sw on the quality of cocoon and fecundity. When spores of the new microsporidia were fed to the fourth instar larvae, immediately after ecdysis, of four strains, such as N124, N132, N134 and C124. the cocoon wt, shell wt, and no of eggs laid became significantly lower than those of the non-treated control.

**Iizuka T (1972). Histo-pathological studis on the midgut epithelium infected with *Streptococcus faecalis* AD-4 in silkworm larvae reared on the artificial diet. *J. Sericult. Sci. Jpn.* 41:327-332.**

Histopathological studies were made on the midgut epithelium after infection with *S. faecalis* AD-4 in silkworm larvae reared on the artificial diet under xenic and axenic conditions. Inoculations were perorally and hypodermically made in the 5$^{th}$ instar larvae. The results obtained were summarized as follows:

1. Bacterial cells attached within peritrophic membrane in three days after peroral inoculation with *S. faecalis* AD-4 and then they gradually multiplied as large colonies at that place. Eventually, peritrophic membrane dissolved in six days after inoculation. As the results, it seemed to obstruct absorption of nutrition in larvae. 2. In the infected larvae, the histological changes of epithelium occurred at first in the goblet cells and then in the cylindrical cells. 3. In the early stage of infection, nuclei of goblet cells hypertrophied and the cytoplasm was deeply stained with pyronine at first. However, the stainability with pyronine decreased in seven days after the inoculation and vacuolizations resulted from the disruption of the cytoplasm. 4. In five days after the inoculation, nuclei of cylindrical cells hypertrophied, chromatin materials in the nucleus became the gathered mass and they gradually dispersed. The cytoplasm became to enlarge and the stainability with pyronine decreased. The spherical body in the cytoplasm of cylindrical cells was observed in five days after the inoculation. The spherical bodies did not show any stainability with pyronine. Size of the spherical bodies was 7 to 15 microns. 6. In epithelium of the larvae hypodermically injected with AD-4, after 24 h, the stainability with pyronine of the goblet cells was already decreased and nuclei of the cylindrical cells hypertrophied. In three days after the infection, the cytoplasm extremely degenerated and chromatin materials in the nucleus disbursed. The spherical body was unrecognized. 7. No cytological changes between epithelium of the healthy larvae reared on mulberry leaves and the artificial diet under xenic and axenic conditions, were microscopically observed.

**Iizuka T (1972). The pathogenic mechanism of the disease caused by *Streptococcus faecalis* AD-4 in silkworm larvae reared on the artificial diet. *J. Sericult. Sci. Jpn.* 41:333-337.**

In the larvae after infection with *S. faecalis* AD-4, while pH value of digestive fluid decreased, pH value of hemolymph increased in four days after inoculation. Both solutions became the same pH value in six days after inoculation. Viable cells were not isolated from hemolymph of the larvae before five days after inoculation, but in six days, numbers of viable cells logarithmically increased. Dead cells of *S. faecalis* AD-4 and the culture solutions filtrated by Seitz's filter were not pathogenic to silkworm larvae, but only viable cells were pathogenic. The pathogenic mechanism of bacterial disease in silkworm larvae reared on the artificial diet was discussed by the results obtained in this paper and in the former paper by Iizuka (1972) and the pathogenic mechanism was postulated as follows:

In the larvae infected with *S. faecalis* Ad-4, bacterial cells multiplied to attach within pertrophic membrane after lag phase for two or three days, and then they gradualllhy multiply as large colonies. By the results, peritrphic membrane are dissolved and goblet cells and cylindrical cells of midgut oare vacuolysed in six days after inoculation. The death in larvae seems to result from what digestive fluid passes through the vacuolysed cells of midgut epithelium into hemolymph and septicemia is caused by the rapid growth of bacterial cells in the hemolymph.

**Ayzawa C (1972). Studies on the infectious flacherie of the silkworm, *Bombyx mori* L. I. Purification of the virus and its some properties. *J. Sericult. Sci. Jpn.* 41:338-344.**

Equilibrium density gradient centrifugation method using CsCl was applied for the purification of the infectious flacherie virus of the silkworm. The reliable results were obtained by this method and some physical properties of the virus were examined.

A distinct band was generally observed by means of the density gradient centrifugation, but in a few cases one more fine band appeared. The optical density of the main band presented at 260 and 280 mμ and uniform particles were observed under the electron microscope using negative staining. Diameter of the virion 26±2 mμ. Buoyant density ($\rho$25) was calculated at 1.375. Sedimentation constant ($S_{20}$) was measured 183.0 with schrieren pattern. Recovery of the virus in the present study was 15.8% at maximum in biological activity.

**Inoue H and Ayzawa C (1972). Studies on the infectious flacherie of the silkworm, *Bombyx mori* L. II. Observation of infected midgut epithelium by fluorescent antibody technique. *J. Sericult. Sci. Jpn.* 41:345-348.**

Flourescent antibody technique was adopted to demonstrate the presence of infectious flacherie virus in the midgut epithelium of silkworm. Specific fluorescence was observed in the cytoplasm of the goblet cell. It was also noticed in the cytoplasm of the columnar cell, but the intense of fluorescence and the number of stained cells were faint and few.

On the smeared preparations of the midgut epithelium at the early stage after infection, stained goblet cells were frequently observed. One or two small light bodies, shown the round or oval shape and the high coloured outline, were found in the cytoplasm of the columnar cell. Isolated small light bodies were found when the cells were separated from the midgut epithelium with trypsin.

**Furuta Y and Ayzawa C (1972). Effects of the duration and times of inoculation of virus on the infection to the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 41:371-374. [Japanese]**

**Inoue H (1972). Studies on the multiplication of infectious flacherie virus in the silkworm, *Bombyx mori. J. Sericult. Sci. Jpn.* 41:437-444.**

Some experiments on the multiplication of IFV in the silkworm were carried out and the observation on the frozen sections of tissues by a fluorescent antibody technique lead to the following conclusions.

The virus first developed in a part of the anterior portion of the midgut. The fluorescent cells, which showed greenish-yellow fluorescence, appeared separately and increased in number with the progress of time, and it was presumed that the virus seldom spread from infected cells to adjacent cells in a direct manner, but frequently through the haemolymph and the midgut lumen. The virus might scarcely multiply in the tissues such as the foregut, hindgut, silk gland, malpighian tubule, trachea, ganglion, brain, integument, fat body, testis and ovary, etc. since the specific fluorescence was not observed in the aforegoing tissues.

The goblet cell of the midgut was the main target cell of the virus and was degenerated following the infection. On the contrary, it was presumed that the columnar cell was not a main target one because specific fluorescence could not be detected in the initial stages of infection. In a progressive stage of the infection, large or small light bodies with specific fluorescence wee recognizable in the cytoplasm of some columnar cells of the $4^{th}$ and $5^{th}$ instar larvae, however, these light bodies might be the degenerated goblet cells, which had been absorbed into the uninfected columnar cells. Some columnar cells contained virus particles, but these particles might be only derived from the light body. In this connection, small light body showed the intense fluorescence, indicating the virus was concentrated according to the degeneration of the goblet cell.

## Vol. 42 1973

**Furuta Y and Ayzawa C (1973). Studies on the infectious flacherie of the silkworm, *Bombyx mori* L. III. Changes in the $LD_{50}$ of the virus passed through different strains of the silkworm. *J. Sericult. Sci. Jpn.* 42:1-10.**

It has been generally recognized that infectious flacherie virus (FV) sampled from the silkworm of f1 hybrid C124×J124, revealed that low $LD_{50}$ (-log) for C124, as a resistant silkworm strain and high $LD_{50}$ (-log) for J124 as a susceptible silkworm strain in *per os* inoculation. However, when FV sampled from C124 (C124FV) inoculated into C124 and J124, LD50 (-log) in the former case was higher than that in the latter. In order to know the mechanism of this phenomenon, experiments were carried out and the results obtained were summarized as follows:

(1) The decrease of $LD_{50}$ (-log) for J124 was immediately shown after the passage of virus through C124 in one generation and the decrease was occurred independently with the times of passage. On the other hand when the virus was passed again through C124×J124 (C124×J124FV) or J124 (J124FV), $LD_{50}$ (-log) was lower for C124 than for J124. (2) The longer the observation period, the smaller difference in $LD_{50}$ (-log) between J124 inoculated with C124FV and C124 inoculated with the same C124FV, though the period of lethal infection in J124 was always longer than that of C124. (3) Suspension of the healthy C124 of J124 larva, prepared with the same method as preparation of virus was mixed with C124FV or J124FV though the period of lethal infection in J124 was always longer that than of C124. (4) When C124FV and J124FV were purified with density gradient method using CsCl, no difference was observed in the shape and effective buoyant density of virus. The results of $LD_{50}$ (-log) test by *per os* inoculation of the purified virus were similar to those by the partially purified virus, but difference in $LD_{50}$ (-log) between C124 and J124 was reduced when the test was done by subcutaneous injection. (5) No interference was observed between C124Fv and J124FV in the inoculation to C124×J124. (6) No obvious difference in the antigenicity of C124FV and J124FV was observed in the neutralization test. (7) Virus multiplication curve for C124 was the same as that for J124 as far as bioassay was concerned with the virus sampled from C124×J124. (8) In order to know whether the afore-going phenomena were observed or not in other silkworm strains, viruses sampled from three resistant strains such as Daizo, Kuroko and C103 from three susceptible strains such as C7, SO and Mus-1 were inoculated into each strain with various combinations. However, no change in $LD_{50}$ (-log) unlike in the case of J124 inoculated with C124FV was detected in those strains.

**Matsui M (1973). Electron microscope study on the flacherie virus infection in the goblet cell of midgut of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 42:11-16.**

Electron microscope observations on the midgut of silkworm larva infected with a flacherie virus indicated that the goblet cell was the main target cell of the virus multiplication: virus particles appeared in the cytoplasm and not in the nucleus of goblet cell.

In an early stage of infection, virus particles appeared accompanying with vesicles which were specifically induced by the virus infection. Moreover, at the adjacent portion to virus-specific vesicles and virus particles, electron dense bodies were also recognized. The virus-specific vesicles were almost round about 100-300 nm in dia and they contained filamentous structures. These two virus specific structures, virus specific vesicle and electron dense body, took place with the virus assembly, suggesting these structures might possess materials necessary for virion formation.

In a late stage of infection, the cytoplasm of goblet cell was filled with virus particles and virus-specific vesicles. The matrix containing the viral coat protein, the proliferation of cisternae and the linear accumulation of high electron dense material were also observed in the cytoplasm. In the nucleus, on the other hand, high electron dense masses appeared in a late stage of infection.

In the last stage of infection, the goblet cell was morphologically changed to be round. The goblet chamber became small in size and mitochondria as well as microvilli changed to show membraneous structures. Because of the disappearance of the plasma membrane, the limiting membrane of the rounded goblet cell was appeared to be a single membrane. In the columnar cell of the infected midgut, inclusion bodies were seen in the cytoplasm. However, their fine structures wee quite similar to those of small rounded goblet cells, indicating inclusion bodies in the columnar cell were probably derived from degenerated goblet cells.

**Yamnaguchi K (1973). Studies on the midgut-nuclear polyhedrosis of the silkworm, *Bombyx mori* L. (VIII) A previously undescribed virus strain, $B_1$. *J. Sericult. Sci. Jpn.* 42:74-78.**

A strain of *Bombyx* midgut-nuclear polyhedrosis virus was isolated from the silkworm larva infected with B strain of the virus. The new virus strain was designated as strain B1. The polyhedron consists of numerous small polyhedra connecting with each other, and it is like a bunch of grapes. The polyhedra are mainly formed in the cytoplasm of cylindrical cells infected with the virus, though some rod shaped bodies are observed in the nucleus of the same cell during the early stage of infection. As the disease advances, however, a few inclusion bodies having the same outline as the polyhedra in the cytoplasm, and tetragonal inclusion bodies are occasionally found in the nucleus.

**Aratake Y (1973). Strain difference of the silkworm, *Bombyx mori* L., in the resistance to a nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 42:230-238.**

A study was made on the strain difference of silkworm, in the resistance to the peroral infection with a NPV. The results indicated that there was a large variation for the resistance among various silkworm strains and the variation also existed among batches of the same strain, suggesting a number of genetical factors might be involved in the resistance of the silkworm to a NPV. Inter strain difference in the resistance of the newly hatched larvae showed a comparable relationship with that of the 3rd instar larvae immediately after ecdysis.

In general, F1 hybrids showed marked heterosis in their resistance to a NPV virus and the heterosis was decreased in F2 hybrids. When female of various silkworm strain were crossed with males of the wild silkworm, *Teophila mandarina*, the resistance of F1 hybrid was varied according to the resistance of their maternal silkworm strains. Resistance of F1 hybrids from a bimolting strain crossed with various tetra-molting strains varied according to the resistance of tetra-molting strains used as one of their parents.

**Yamaguchi K and Hukuhara T (1973). Studies on the midgut-nuclear polyhedrosis of the silkworm, *Bombyx mori* L. (IX) New virus strain, $B_2$. *J. Sericult. Sci. Jpn.* 42:239-243. [Japanese]**

**Aratake Y and Ueno H (1973). Inactivation of a nuclear polyhedrosis virus by the gut-juice of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 42:279-284.**

It has been known that a NPV is inactivated *in vitro* by the gut juice of silkworm larva. The antiviral activity of the gut-juice obtained from 5th instar larva was higher than that of the 4th instar larva. As for the same instar larvae at the late stage showed much higher antiviral activity

of the gut-juice that at an early stage. Although the activity of the gut juice was varied with silkworm strains and the seasons of larval rearing, the gut-juice obtained from larvae of spring rearing showed the highest activity. The activities of the gut-juice obtained from F1 hybrids were much higher, in general, than those from their parental inbred strains, back crossed hybrids and F1 hybrids. No change of activity was detected in the gut-juice which had been stocked at -20°C for 1½ years.

**Furuta Y (1973). Studies on the previously unreported flacherie virus infecting the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 42:443-453. [Japanese]**

## Vol. 43 1974

**Watanabe H (1974). Electron microscope investigation on dissolution of polyhedra in the gut juice of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 43:29-34.**

Alteration in the ultrastructure of the polyhedron and the virions exposed to the gut juice of the silkworm *in vivo* was studied. Although some nuclear polyhedra were intact after 20 min. exposure, the majority of polyhedra revealed that channel like dissolutions or fissures were produced inside and conspicuous dissolutions of polyhedron protein in regions around the embedded virions were also occurred. Longer periods of exposure to the gut juice resulted in fragmentation of polyhedra and each free polyhedron fragment was further dissolved to release the free virions. However, the electron dense outer layer of polyhedron was almost intact even after complete dissolution of the inner part of polyhedron. It was the most commonly observed that both the virions embedded in the dissolving polyhedra and the virions released from the polyhedra were affected by the gut juice to be released the outer and inner membranes from the nucleocapsids. In contrast, cytoplasmic polyhedra treated with the gut juice were solubilized progressively from the periphery to the inner part, releasing virions one by one. Thus the increasing periods of exposure of cytoplasmic polyhedra to the gut juice caused an increase of free virions and a decrease of polyhedra size.

**Aratake Y, Kayamura T and Watanabe H (1974). Inactivation of a cytoplasmic polyhedrosis virus by gut-juice of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 43:41-44.**

Inactivation tests of a cytoplasmic polyhedrosis virus by gut-juice of the silkworm, *Bombyx mori* were carried out in the present study. Although every gut juice from the $5^{th}$ instar larvae of 14 silkworm strains showed the antiviral activity, a large inter strain difference in the activity was involved. Inasmuch as not a fairly high correlation was observed between the inter strain difference in resistance to peroral infection of the virus and antiviral activity of the gut juice, it would be reasonable to draw a conclusion that the mechanism of host resistance to peroral infection with the virus depends in part, but not in all, on the antiviral activity of the gut juice.

**Iizuka T (1974). Antibacterial activity in digestive juice of silkworm larvae and in substances extracted from feces. *J. Sericult. Sci. Jpn.* 43:89-93.**

Bacterial numbers of *Streptococcus faecalis* AD-4 abnormally increase from $10^9$ to $10^{13}$/ml in the midgut of larvae of the silkworm reared on the artificial diet, and silkworm larvae die at all. However, numbers of *S. faecalis* AD-4 do not increase over $10^7$/ml level in the midgut of larvae reared on mulberry leaves. Therefore, it is postulated that there is some antibacterial substance in the mulberry leaves, or that there is some antibacterial substances translated from substances included in mulberry leaves in the digestive juice. In this experiment, the antibacterial activity of some substances translated from substances included in mulberry leaves in the digestive juice.

Antibacterial activity was found in digestive juice of larvae, sodium copper chlorophillin solution and crude chlorophyll extracted from silkworm feces. However, antibacterial activity in untreated juice of mulberry leaves was not recognized and also in red fluorescent protein which inactivity to nuclear polyhedrosis virus was found by HAYASHIYA *et al*. (1970), antibacterial activity was not recognized.

**Watanabe H, Tanaka S and Simizu T (1974). Inter strain difference in the resistance of the silkworm, *Bombyx mori*, to a flacherie and a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 43:98-100. [Japanese]**

**Aratake Y and Kayamura T (1974). A test of virus dispersion and the development of epizootic in a laboratory population of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 43:150-156. [Japanese]**

**Kanke E (1974). Electrophoretic changes of haemolymph protein of silkworm, *Bombyx mori* L., infected with the infectious virus. *J. Sericult. Sci. Jpn.* 43:171-174.**

Haemolymph protein of $5^{th}$ instar larvae of the silkworm infected with the infectious flacherie virus was subjected to electrophoresis in agarose gel. Electrophoretic pattern in healthy larvae showed 3 to 4 bands in a silkworm strain of Shungetsu and 5 to 6 bands in a hybrid between strains of Kokko and Seihakum respectively. In virus infected larvae, however, decrease or disappearance of protein bands was observed distinctly with the advancement of the disease. Protein bands with low mobility disappeared in early time of the infection. There was little difference between male and female in these changes of Haemolymph protein and a defect of production of female protein in infected female larvae.

**Kurisu K and Himeno M (1974). Pathogenicity of the virus-like particles isolated from the silkworm, *Bombyx mori*, infected with a flacherie virus. *J. Sericult. Sci. Jpn.* 43:195-199.**

Flacherie virus particles were separated into the FV1 and FVII (HIMENO *et al*, 1973). FVI corresponds to the flacherie virus reported by AYUZAWA (1972). This paper deals with the pathogenicity of FVI and FVII to silkworm larvae. The results indicated that the larvae inoculated with FVII, eith *per os* or intrahemo-coelically, showed similar syndrome and mortality to those of the larvae infected with FVI. Moreover, the larvae inoculated with FVII produced only FVII particle. These results suggest that the FVII particle is a virus and the pathogenicity is similar to that of FVI.

**Abe Y and Ayuzawa C (1974). Susceptibility of larval tissues in the silkworm, *Bombyx mori* L., to the nuclear polyhedrosis virus. I. Changes of the susceptibility to the virus of mid-gut epithelial cells in the silkworm larvae treated with low temperature. *J. Sericult. Sci. Jpn.* 43:200-205.**

Changes of the susceptibility of midgut epithelium in the silkworm larvae treated with low temperature to the nuclear polyhedrosis virus were studied by microscopical observation. When the larvae were chilled at 5°C for 24 h just after the moulting without any feeding and inoculated with the polyhedra, the posterior portion of midgut in these larvae was heavily affected with virus, specific C.P.E. and polyhedra were formed in both cylindrical cells and goblet cells, but in anterior portion of midgut, infected cells could hardly be seen. Most of larval body in these insect became shorter and died within 24-48h after the inoculation of polyhedra.

In larvae which were chilled after the feeding following the moulting and non-chilled larvae, polyhedral bodied could not be seen in both cylindrical and goblet cells. Most of these larvae died over of 72 h after the inoculation of polyhedra, showing the swelling of segmental membrane. From these results, it was concluded that, usually cylindrical cells and goblet cells of

midgut epithelium in the silkworm are not so susceptible to the nuclear polyhedrosis virus, but if the larvae are chilled at 5°C after the moulting without any feeding, the susceptibility of both cylindrical cells and goblet cells become higher.

**Fujii M (1974). Poisoning symptoms of silkworm larvae, *Bombyx mori* L., by the oral application of fluorine compound. *J. Sericult. Sci. Jpn.* 43:236-240.**

Physiological damages caused by the oral administration of fluorine in *Bombyx mori* were investigated. Results obtained are summarized. Alimentary canal of the larvae damaged by the fluorine became light green or light brown containing little mulberry leaves and the larvae exhibited light brown. The growth and molting of such larvae were delayed, indicating a symptom similar to that of flacherie. When larvae were affected by the fluorine, the increasing rate in body weight declined at first, followed by the reduction in amount of food ingested and then their development prolonged. In chemical analyses, ingested fluorine was mostly retained in alimentary canal, digestive juice and blood and was very little found in integument and silk gland. From these results, it may be presumed that the death of silkworm larvae caused by the oral administration of fluorine can be mainly attributed to physiological damages of alimentary canal caused by the fluorine toxicity, because myuch amount of fluorine is accumulated in the wall of alimentary canal.

**Kurata K and Takahashi S (1974). An assay of formaldehyde gas concentration in the silkworm rearing room sprayed with formalin solution. *J. Sericult. Sci. Jpn.* 43:245-249.**

The concentration of formaldehyde gas in the silkworm rearing room sprayed with 3% formalin or fumigated with Neo PPS was determined by the use of 2-hydrazinobenzothiazol. Just after the spray of the formalin solution, the concentration of formaldehyde gas ranged from 250 to 300 μg per a liter of air. With the time lapse after the spray, the concentration decreased and about 40 μg per a liter of air after 24 h. When Neo PPS (Para formaldehyde) was fumigated, the conentration was about 600 μg per a liter of air just after the disinfection and decreased rapidly to about 60 μg per a liter of air after 16 h. It was found from the experiment that the concentration of formaldehyde gas in air was increased in accordance with the increase in the concentration of formalin solution sprayed at varying temperature between 20 to 28°C.

**Kurisu K (1974). Lethal effect of secondary bacterial infection on silkworm larvae infected with flacherie virus. *J. Sericult. Sci. Jpn.* 43:277-282.**

From histopathological view points, the author has classified the symptomatic diversity witch appeared in silkworm larvae infected with flacherie virus and reared with mulberry leaves. It was considered that the mortality in viral flacherie might result from the dynamic interrelationship among the virus invasion, the renewability on the midgut epithelium and the occurrence of secondary bacteriosis. Present experiments were carried out to determine the lethal effect of bacteriosis caused by the bacterial contamination through mulberry leaves *per os*.

The mortality in the mixed infection with virus and bacteria invading simultaneously, was the highest. But in the natural double infection, virus infection happened primarily and the secondary bacterial infection occurred through mulberry leaves *per os*, the lethal effect was moderated. In the case of the silkworm reared under the aseptic condition, where the virus invasion proceeded only and the bacterial infection did not occur, the mortality of the gnotobiotic larvae became lower. When these gnotobiotic larvae were transferred into the bacterial contamination by rearing them with mulberry leaves, the mortality of the larvae increased rapidly. Meanwhile the mortality in the natural double infection was depressed remarkably by feeding of antibiotic substances. The difference of histopathological observations between above mentioned gnotobiotic larvae and ordinary ones was indicated as the distinct contrast in the occurrence of secondary bacteriosis. Thus the mortality in viral flacherie under the rearing with

mulberry leaves was considered to be caused by the secondary bacteriosis which had been predisposed by the primary virus infection.

**Kurisu K and Matsumoto T (1974). Histopathological observations on the midgut epithelium in the germ-free silkworm larvae infected with flacherie virus. *J. Sericult. Sci. Jpn.* 43:283-289.**

The newly moulted silkworm larvae of the 3rd instar which were reared aseptically were infected with the flacherie virus perorally and the process f histopathological changes in the midgut epithelium was investigated. The results were summarized as follows:

(1) As the first pathological changes, goblet cells became pyronine positive, shrinked and globular. Then the basiophilic inclusion bodies appeared in the cylindrical cells. (2) At the later half of the 4th instar, the falling-off of the diseased cylindrical cells into the midgut lumen became apparent. At the same time, following pathological changes were observed as the symptoms of advanced state, goblet cell was not observed and also inclusion bodies were not noticeable in the cylindrical cells. (3) During the moulting, the total or partial replacements of the midgut epithelium caused by the regeneration cells were observed. Some supplemented regeneration cells differentiated into the goblet cells and the pathological changes in the midgut epithelium were improved. So the above mentioned renewability might be the one of the defending mechanisms against the flacherie virus invasion. (4) In the midgut epithelium of the non-moulting larvae, in which the renewability by the regeneration cells was not observed and goblet cells did not exist, pyronin-stainable granules with 1~5μ in diameter were observed in the cylindrical cells. These basiophilic granules were different from the inclusion bodies reported by IWASHITA (1964) and they were thought to be derived from the nucleus of the cylindrical cell.

**Kurisu K (1974). Histopathological changes and appearances of pyroninophilic granules in the cylindrical cell of the silkworm larvae infected with flacherie virus. *J. Sericult. Sci. Jpn.* 43:290-295.**

On studying about the histopathological progress in the virus-invaded midgut epithelium, it was speculated that the cylindrical cell had some relation with the multiplication of flacherie virus (KURISU and MATSUMOTO, 1974). This report is concerned with the histopthological changes in the cylindrical cell of silkworm larvae infected with flacherie virus and appearances of pyronine stainable fine granules in it. The results are summarized as follows:

(1) The pyronine stainable substances were produced and accumulated in the nucleus of the cylindrical cell, according the progress of the flacherie virus invasion. (2) In the cytoplasm of the cylindrical cell, there appeared the pyrnine stainable granules 0.6~0.8 μ or occasionally about 1 μ in diameter, they were different from the pyronine stainable inclusion bodies 3~10 μ in diameter reported by IWASHITA (1965). (3) Considering the histopatholgical specificities, production and acumultion of the pyronine stainable substances in the nucleus of the cylindrical cell, the appearances of the pyronine stainable fine granules in the cytoplasm of it and the characteristic histological progress in the virus invaded midgut epithelium (KURISU and MATSUMOTO, 1974), it was considered that the multiplication of flacherie virus occurred in the cylindrical cell and the pyronine stainable fine granules seemed to be the elementary bodies, which indicted the virus multiplication photo-microscopically. (4) Flacherie virus was purified into the two sorts of spherical particles by HIMENO et al, (1974) and each of them was virulent to silkworm larvae (KURISU and HIMENO, 1973). Though the flacherie virus multiplication in the cylindrical cell was denied by INOUE (1972), the virus antigen used by his fluorescent antibody technique seemed to be 180S virus (AYZAWA, 1972 and HIMENO et al, 1973). The results of this experiment might be to indicate the multiplicaion of 13S virus or the last stage of virus invasion.

**Furuta Y and Ayuzawa C (1974). Studies on the infectious flacherie of the silkworm, *Bombyx mori* L. IV Pathological research of the disease occurred under the aseptic and non-aseptic conditions. *J. Sericult. Sci. Jpn.* 43:311-317. [Japanese]**

**Inoue H (1974). Multiplication of an infectious flacherie virus in the resistant and susceptible strains of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 43:318-324.**

The multiplication of an infectious flacherie virus (IFV) in the susceptible and the resistant strains of the silkworm, *Bombyx mori*, were investigated by means of the fluorescent antibody method, which demonstrated the presence of IFV in the frozen sections of diseased midguts.

If the peroral inoculation test of IFV to newly hatched larvae, all the larvae of susceptible strains died by the 3rd instar, whereas most of the larvae of resistant strains were alive and pupated. However, the fluorescent antibody tests revealed that IFV multiplied well in the same degree both in larvae of the susceptible and resistant strains. In the susceptible larvae, the number of cells with fluorescence increased just prior to death, while in the resistant larvae which were alive to pupate, the number of fluorescent cells also increased, but were much reduced temporarily just after every moulting. The temporal reduction of the number of fluorescent cells during the IFV infection in the resistant larvae indicated that the infected goblet cells were discharged into the gut lumen at every moulting and the regenerative cells located in *nidi* of the midgut developed into new goblet cells for the physiological repair. Therefore, the regenerative ability of the midgut cells might be mainly concerned with the resistance of the silkworm larvae to the lethal infection of IFV.

**Furuta Y and Ayuzawa C (1974). Studies on the infectious flacherie of the silkworm, *Bombyx mori* L. V Changes in the infectivity of the virus passed through different strains of the silkworm (ii). *J. Sericult. Sci. Jpn.* 43:332-336. [Japanese]**

**Inoue K (1974). Phagocytosis of nuclear polyhedra and the localization of the phosphatase in the granular cell of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 43:394-400.**

When larvae of the silkworm were injected with nuclear polyhedra, the granular cells (phagocytic leucocytes) phagocytosed the polyhedra and enclosed them into a phagosome. In an early stage of phagocytosis, polyhedra were morphologically intact and acid phosphatase (AcPase) activity was observed in the phagosome. But in the advanced stage of phagocytosis, when the pagosome had been fused with the lysosome, polyhedra were transformed into spheres and AcPase activity was recognized not only on the external surface and internal matrix of polyhedra, but also on the occluded virions. Subsequently, the virions were degraded and there were large number of vacant spaces where virions had been occluded in the phagocytosed polyhedra. Such degraded polyhedra were frequently observed outside of the granular cell. These results suggested that the phagocytosed polyhedra and their occluded virions were digested by lysosomal enzymes.

**Furuta Y (1974). Studies on the previously unreported flacherie virus infecting the silkworm, *Bombyx mori*. II. The infectivity to the several strains of the silkworm and serological characteristics of the virus. *J. Sericult. Sci. Jpn.* 43:405-411. [Japanese]**

**Matsui M and Watanabe H (1974). Electron microscope observation on the midgut of *Theophila mandarina* infected with a flacherie virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 43:467-470.**

Electron microscopic observations were made on the midgut of *Theophila mandarina* larvae infected with a flacherie virus of the silkworm, *Bombyx mori*. In the goblet cell, a number of virus specific vesicles were formed in the cytoplasm accompanying with the virogenesis. And

filamentous structure, probably viral RNA, were observed within the vesicles. In the infected columnar cell, on the other hand, mitochondria swelled and became round. In the periphery of mitochondria, many small vesicles (peripheral vesicles) emerged and filamentous structures were also seen within them. Thus, the development of the peripheral vesicles seemed to be closely related to the multiplication of a flacherie virus.

**Nagae T (1974). The pathogenicity of *Streptococcus* bacteria isolated from the silkworm reared on an artificial diet. I. Difference in the pathogenicity of the bacteria to silkworm larvae reared on an artificial diet and to those reared on mulberry leaves. *J. Sericult. Sci. Jpn.* 43:471-477.**

Eight strains of *Streptococcus* bacteria were isolated from diseased silkworm larvae reared on an artificial diet. All the bacterial strains were highly pathogenic to silkworm larvae reared on an artificial diet. The symptom of this disease appeared in the 4$^{th}$ and 5$^{th}$ larval instars. Enormous bacterial multiplication was observed in the mid guts. The same bacterial strains were not pathogenic to silkworm larvae reared on mulberry leaves. The bacteria ingested by these larvae, scarcely multiplied in their mid guts.

## Vol. 44 1975

**Kurisu K, Matsumoto T and Inoue Y (1975). Enhanced multiplication of enterococcus in the mid-gut rumen of the starved silkworm larvae, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 44:7-10.**

The multiplication of bacteria in the mid-gut rumen of the silkworm larvae, when they were starved or ligatured, was investigated. In the mid-gut rumen of the healthy silkworm larvae of the 5$^{th}$ instar, the number of general bacteria increased at the initial half of the 5$^{th}$ instar and decreased at the later half. Such mode of increase and decrease, however, were not observed in the case of *enterococcus*.

When food substances in the mid-gut rumen were not allowed to excrete for 24h by the ligature of the larval posterior portion, enhanced multiplications of general bacteria and *enterococcus* were observed. In such circumstance, the multiplication of *enterococcus* was more intensive than that of general bacteria. Under the starving condition, the number of general bacteria decreased exponentially, but the number of *enterococcus* increased remarkably.

**Miyagawa M (1975). Effect of rearing temperature on the formation of nuclear inclusion in the midgut of the silkworm, *Bombyx mori* infected with a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 44:26-32.**

It has been reported that the midgut of the silkworm infected with a CPV frequently contained columnar cells with nuclear inclusion. In the present study, the effect of rearing temp on the formation of nuclear inclusion was investigated.

When the rearing temp after the virus inoculation to the larvae was low (15-20°C), the frequency of columnar cells containing nuclear inclusions was much higher and more inclusion was formed than in the case of rearing at the ordinary temp (25°C). The nuclear inclusion formation under low temperature rearing took place prior to the polyhedron formation in the cytoplasm; under the ordinary temp rearing the nuclear inclusion and the polyhedra formed at the same time.

The nuclear inclusions were tetragonal in outline at an early stage of inclusion, but they gradually changed their shape as the disease proceeded, and, in turn, exhibited hexagonal outline

at a late stage of infection. Difference in the methods of virus inclusion and the feed had no apparent effect on the formation of the nuclear inclusion in the columnar cells.

**Kawakami K (1975). Susceptibility of several varieties of the silkworm, *Bombyx mori* L. to *Aspergillus* disease and germination of fungus spores in larval haemolymph. *J. Sericult. Sci. Jpn.* 44:39-44.**

Two formalin resistant isolates (K1, S85) of *Aspergillus flavus* LINK were used as *Aspergillus* disease agent. Silkworm larvae of both the 2nd and 4th instar of three varieties of Japanese races, J124, J131, J134 and two varieties of Chinese races C124, C135 were used as test object for the inoculation of serial dilution of the spore suspensions. The results indicated that Chinese races were more resistant than Japanese ones to the infection with *Aspergillus* fungi.

On the other hand, in the germination test of conidia in haemolymph obtained from larvae of each variety of the silkworm, no difference among these varieties were detected in the percentages of germination and the length of germ tubes of the incubated conidia.

It is concluded that the degree of the susceptibility to *Aspergillus* infection among different varieties of the silkworm are unproportional to the germination rate or the length of the germ tubes of conidia in the host haemolymph and the germination test cannot be used to determine the susceptibility of silkworm varieties.

**Shimizu T (1975). Pathogenicity of an infectious flacherie virus of the silkworm *Bombyx mori*, obtained from Sericultural farms in the suburbs of Ina city. *J. Sericult. Sci. Jpn.* 44:45-48. [Japanese]**

**Iizuka T, Koike S and Mizutani JT (1975). Antibacterial substances in feces of silkworm larvae reared on mulberry leaves. I. Antibacterial activity of protocatechuic acid and *p*-hydroxybenzoic acid isolated from feces. *J. Sericult. Sci. Jpn.* 44:125-130.**

In this paper we report on the antibacterial activity of procatechuic acid and p-hydroxybenzoic acid isolated as the main antibacterial substances from feces. The fractionation of the dried and powdered feces was carried out. A marked antibacterial activity was observed in the ethyl acetate extractable fraction. This fraction was subjected to a silica gel column chromatography. The most of biological activity was concentrated in the effluent from the column with benzene: MeOH: AcOH (88: 10: 2, v/v). The most active effluent was then subjected to a polyamide C-200 column chromatography. As the result, two main spots on a polyamide B-O thin layer chromatography with antibacterial activity were observed. These two substances which gave blue colour (B1) and violet colour (V1) under UV ray were isolated and then B1 was procatechuic acid and V1 as p-hydrobenzoinc acid, respectively.

Antibacterial activity of some related phenol compounds including these two substances isolated feces was tested. In some compounds tested, hydroquinone, o-vanillin and pyrogallol strongly disturbed the growth of *S. faecalis* AD-4. Only scopolin out of several phenolic compounds found in mulberry leaves showed antibacterial activity.

**Kurisu K, Matsumoto T, Himeno M and Tomioka Y (1975). Immunochemical properties of the flacherie viruses in the silkworm, *Bombyx mori* L. I. Neutralization test. *J. Sericult. Sci. Jpn.* 44:151-153.**

Antisera against the two flacherie viruses (FVSI and FVSII) with the different sedimentation coefficient were prepared in the albino rabbits, and the following immunochemical properties were obtained. In the neutralization tests, both viruses were neutralized by the corresponding antiserum. In the cross reacting tests of FVSI of FVSII antiserum against the other virus, the

neutralizing activities were similar and a precipitation band was recognized in each reaction by the OAKLEY-FURTHROPE's method. These evidences suggest that both viruses serologically resemble each other and they have a common antigen.

**Ishihara R (1975). Effect of *Nosema bombycis* infection on the termination of diapause of the silkworm eggs and on the serosa cells. *J. Sericult. Sci. Jpn.* 44:165-166. [Japanese]**

**Kurisu K (1975). Penetration of muscrardine hyphae through the hair socket of the skin and the side-wall of the tracheal manifold in the silkworm larvae. *J. Sericult. Sci. Jpn.* 44:207-211.**

In the case of hyphal penetration of muscardine fungi through the body-wall of silkworm larva, the hair-socket of the skin and the side wall of the tracheal manifold were considered to be the weak points against hyphal invasion.

In the $5^{th}$ instar larvae, the connecting part of the seta and its formative cell of the skin had a thin cuticular layer, and the space b/w the base of the seta and the papilla of the cuticle was estimated 2~3μm in width. In this particular part of the skin, the histopathological changes indicating the pre invasional and intensive fungus penetration were frequently observed as well as the defense response against the fungal invasions. And such phenomena were less frequently seen in the other parts of the skin.

The side-wall epithelium of tracheal manifold in the stigma of larva was covered by a thin cuticular layer, and placed under the hiatus and the lamella pores of the sieve plates. In this part, the histopathological changes to indicate the intensive fungus penetration were observed in the larvae treated at the lethal inoculation; but in the case of nonlethal inoculation with the weak pathogenic fungi, the visible defense responses of larvae were observed.

After the death of the host larvae, the hyphal appearing from both the seta-setting points and stigmata took place earlier than the other parts of the skin.

**Matsuzaki K and Yoshida M (1975). Patterns of SDS-gel electrophoresis of proteins in haemolymph of the NPV infected silkworm. *J. Sericult. Sci. Jpn.* 44:229-230. [Japanese]**

**Kawakami K, Ebihara T, Tsukida K, Morii K, Ono K and Furusawa T (1975). On the easy detection method for *Aspergillus* fungi in silkworm rearing house by means of the stamp agar. *J. Sericult. Sci. Jpn.* 44:327-332. [Japanese]**

**Kurisu K (1975). Hostopathological observations on the cytomata which appeared in the silkworm larvae infected with flacherie viruses. *J. Sericult. Sci. Jpn.* 44:340-344.**

Tumor-like histopathological changes were observed in the moribund larvae which were infected with flacherie viruses and reared in an aseptic condition. All of these abnormalities were blastemic and belonged to the pathological growths.

Two sorts of tumor like pathological changes wee recognized in the midgut epithelium. Each of them was characterized as papilliformed and inflammatory. The papilliformed cytoma was appeared frequently in the anterior part of the midgut epithelium. Small cells flocked together in this nideleius and goblet cells did not exist. This abnormality appeared as exerting towards the coelon-side at first and finally changed to the visible risings of the midgut epithelium. This abnormality was fairly constricted in a vivisection and an intensive cellular multiplication was recognized histologically.

Besides, tumor-liked histological changes were observed in the coelon. These pathological growths were variable but seemed similarly to be adhesive, histologically. Myoblastoma-liked abnormality, intensive cellular hypertrophy of a tracheal epithelium, thready or flocked unknown tissues and large cells as a macrophage were observed. But the origin of these adhesive cytomata was not identified.

**Aoki J, Yanase K and Kusida T (1975). The pathogenicity to the silkworm and taxonomic considerations on some muscardine fungi. *J. Sericult. Sci. Jpn.* 44:365-370. [Japanese]**

**Abe Y (1975). Dissolution process of nuclear polyhedral bodies in the gut of chilled larvae of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 44:371-374.**

The dissolution of polyhedra of NPV in starved and chilled larvae one day after the first ecdysis was observed histologically. Surface of nuclear polyhera found in the fore-gut and the anterior portion of the midgut of the test animal lost its affinity for acid stain and several slits were observed on mot polyhedra. Inner part of these slits showed high affinity for acid stain. In non chilled control, polyhedra were observed only in the fore-gut and the anterior portion of the midgut. On the other hand, in chilled larvae, polyhedra were observed in all the regions of gut. Inner part of polyhedra observed in both midgut and hind-gut in chilled larvae dissolved and those surfaces lost its affinity for acid stain.

From these results, it is suggested that nuclear polyhedra dissolved immediately after ingested in the fore-gut and the anterior portion of midgut in non chilled control, but the dissolution activity of digestive juice for polyhedra became lower in the case of chilled larvae, and surface membrane of these plyhedra remained insoluble.

**Furuta Y (1975). Studies on the previously unreported flacherie virus infecting the silkworm, *Bombyx mori*. III. Serological difference between the small flacherie virus and the infectious one. *J. Sericult. Sci. Jpn.* 44:375-380. [Japanese]**

**Abe Y (1975). Susceptibility of larval tissues of the silkworm, *Bombyx mori* L., to the nuclear polyhedrosis virus. II. Hisotopathological observations of flacherie-like larvae inoculated with nuclear polyhedra following the low temperature treatment. *J. Sericult. Sci. Jpn.* 44:424-427.**

When the 5$^{th}$ instar larvae inoculated with high concentration of NPV following cold treatment immediately after the moulting without any feeding, these larvae showed the flacherie like symptom, such as shortening body, and death at about 48h after inoculation. But when the larvae were inoculated with low concentration of polyhedral bodies following the same cold treatment, they showed the typical signs of nuclear polyhedrosis such as swelling of segmental membrane and died after 120h or more following the inoculation.

On the other hand, in midgut epithelium of these larvae which showed flacherie like symptom, many number of cylindrical cells and goblet cells were heavily affected with nuclear polyhedrosis virus and infected cells were degenerated and liberated into midgut lumen. Then a lot of bacteria were observed in midgut lumen and hemocoel of these heavily infected, flacherie like larvae. These bacteria were similar to *Streptococcus* group.

From aforegoing results, the process causing the flacherie like symptom was supposed as follows: when the silkworm larvae were exposed at 5°C immediately after the moulting, susceptibility of midgut epithelium to NPV became higher, and the cells of midgut epithelium were heavily affected with the virus by inoculation of high concentration of polyhedra. Infected midgut epithelial cells were degenerated and liberated into midgut lumen. The infected larva died in early time following the secondary invasion of streptococcus like bacteria.

**Yamaguchi K (1975). Studies on the interference between viruses in the silkworm, *Bombyx mori* L. III. Interference between A and $C_1$ strains of the cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 44:468-471.**

Studies have been carried out on the interference between A strain which forms large cubic polyhedra and $C_1$ strain which forms large irregular shaped inclusions of the CPV in silkworm.

When both A and C1 viruses were fed together to silkworm larvae, almost all of the larvae were infected with the two viruses. Although the majority of the midgut cells of the larvae infected with the two viruses contained either A polyhedra or $C_1$ inclusions, the remaining cells contained two types of inclusions, A and $C_1$. When the two viruses were fed to the larvae at 24 h time intervals, interference took place b/w A and $C_1$ viruses at the organismic level, but not at the cellular leel.

**Kurisu K and Tomioka Y (1975). Bacterial penetration through the colon epithelium in the silkworm larvae infected with flacherie virus. *J. Sericult. Sci. Jpn.* 44:491-492. [Japanese]**

**Watanabe H (1975). Variation in the number of nucleocapsid within the envelope of nuclear-polyhedrosis virus multiplied in different tissues of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 44:497-498. [Japanese]**

## Vol. 45 1976

**Seki H and Sekijima Y (1976). Detection of the specific antigen of the infectious flacherie virus in the silkworm, *Bombyx mori* L., by the single radial immunodiffusion method. *J. Sericult. Sci. Jpn.* 45:13-18.**

The experiments were carried out in order to detect the specific antigen of the infectious flacherie virus (IFV) and also to find the optimum condition in which the single radial immunodiffusion method should be applied. The results obtained were as follows. The IFV specific antigen could be detected through the single radial immunodiffusion mehod, by observing a precipitated ring formed around the circumference of the well into which the solution containing antigen was poured. Then antigen-antibody reaction was carried out at 30°C for 24 to 48h and the precipitated ring thus formed was observed by means of the immuno viewer.

The concentration of the anti-IFV serum to be contained into the agar gel plate was 0.25 to 0.5% against the upper layer of the IFV antigen solution extracted crudely and was 1.0 to 1.5% against the upper layer of the IFV antigen solution extracted crudely and was 1.0 to 1.5% against the partially purified IFV antigen substance. It was recognized that there is a linear relationship b/w the size of the precipitated ring and the concentration of the IFV antigen. Therefore, this method is applicable to determine approximately the amount of the IFV antigen from the silkworm larvae infected with IFV.

**Watanabe H, Maeda S, Matsui M and Shimizu T (1976). Histopathology of the midgut epithelium of the silkworm, *Bombyx mori*, infected with a newly isolated virus form the flacherie-diseased larvae. *J. Sericult. Sci. Jpn.* 45:29-34.**

The virus isolated from the suburbs of Ina City, was studied for its hitopathological aspects. The virus is a spherical particle with a diameter of 21 nm and its multiples in the nucleus of columnar cell of the midgut. In addition, a light radioautographic study revealed that DNA synthesis occurred predominantly in the infected nucleus of columnar cell during the virus multiplication. The evidence suggests that the virus is quite similar in histopathological characteristics to the densonucleosis virus of *Galleria mellonella* and animal viruses belonging to the parvovirus group.

**Nagae T and Suzuki T (1976). The pathogenicity of *Streptococcus* bacteria isolated from the silkworm reared on an artificial diet. II. Bacteriological characteristics of the isolated *Streptococcus* bacteria. *J. Sericult. Sci. Jpn.* 45:41-47.**

The strains belonged to *Streptococcus faecium* type and *Intermediate* type were pathogenic to silkworm larvae reared on artificial diet. Two strains of *Streptococcus faecium* of type culture were not at all pathogenic.

**Yamaguchi K (1976). Natural cure of the fall worm, *Hyphantria cunea*, infected with the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 45:60-65.**

Susceptibilities of the fall webworm larvae to several strains of the silkworm CPV were tested, and the process of histological changes in the midgut epithelium infected with the virus was investigated. Although the fall webworm larvae have marked resistance to the silkworm CPV, almost all of the larvae were infected with the virus when the newly hatched larvae were inoculated with a high concentration of virus, and abundant polyhedra were formed in the midgut epithelial cells at the last stage of the 1st instar. The polyhedra formed were the same shape as that tin the silkworm larvae.

During the 1st molting stage, the majority of the infected cylindrical cells were discharged into the gut lumen, and, at the same time, the regenerative cells developed into new epithelial cells for the physiological repair in the extensive part of midgut epithelium. Polyhedra were not formed in the new epithelial cells in the 2nd instar larvae, and no polyhedra were detected through the 3rd instar to pupation. Thus the fall webworm larvae fed a high concentration of the silkworm CPV were alive and pupated. From the results it was recognized that the fall webworm larvae infected with the BmCPV recovered from the infection naturally.

**Kawase S and Kang SK (1976). On the nucleic acid of a newly-isolated virus from the flacherie diseased silkworm larvae. *J. Sericult. Sci. Jpn.* 45:87-88. [Japanese]**

**Miyajima S (1976). Ultraviolet absorption as a titration method of the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 45:89-90. [English]**

Ultraviolet absorbency at 260 nm has been used for a rapid and simple method of determine CPV concentration. The method will detect the non-infective and aggregated virus as well as the active virus.

**Miyajima S (1976). Serological properties of cytoplasmic-polyhedrosis virus of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 45:245-250.**

Experiments concerning precipitation ring test, cross-absorption test and neutralization test for CPV, NPV, IFV and RDV (rice dwarf virus). Precipitin ring, cross absorption and neutralization tests revealed that two strains of CPV, TC and HC which form polyhedra of tetragonal and hexagonal shape respectively had no heterogenic antigens. In the precipitin ring and cross-absorption test, no common antigen of CPV was detected in NPV, FV and RDV. Therefore, it can be concluded that CPV is serologically unrelated to NPV, FV and RDV.

**Miyajima S (1976). Resistance of cytoplasmic-polyhedrosis virus to some physico-chemical treatments. *J. Sericult. Sci. Jpn.* 45:251-257.**

The CPV of the silkworm lost its infectivity when heated at 85°C or at high temp. The critical condition of the virus inactivation was estimated as 85°C for 10 min. in 0.01 M phosphate buffered saline (pH 6.8). The virus was stable at 50°C for 30 min. CPV is a heat resistant virus.

CPV was freeze thawed in a freezer at -20°C and a desiccator at 20°C. No loss of infectivity occurred after 9 cycles of freeze thawing treatment. CPV was not inactivated by ether, sodium deoxycholate and acid (at pH 3.0), which indicates that the CPV belongs to the ether, sodium deoxycholate and acid-resistant group virus.

**Miyajima S (1976). Measurement for titration of the cytoplasmic-polyhedrosis virus concentration by the use of some immunological methods. *J. Sericult. Sci. Jpn.* 45:300-304.**

In order to find the suitable titration method of the BmCPV several immunological methods, such as hemagglutination reaction, flocculation test, slide flocculation test, precipitin ring test and fluorescent antibody method were applied. Relative sensitivity of different methods was examined. The fluorescent antibody method was most sensitive to detect the virus antigen, and the precipiting ring test was the next. The latter was more sensitive that both slide flocculation test and hemagglutination reaction for the detection of the virus. And then the flocculation test was most insensitive among these methods.

The value for the sensitivity of various methods of virus detection indicated that fluorescent antibody method could show up as little as $2 \times 10^{-8}$ mg/ml of the virus. The precipitin ring test, slide flocculation test, hemagglutination reaction and the flocculation test allowed the detection of as little as $6 \times 10^{-4}$ mg/ml, $5 \times 10^{-3}$ mg/ml, $4 \times 10^{-3}$ mg/ml and $2 \times 10^{-2}$ mg/ml respectively. From the results it is considered that the virus titration can be obtained by immunological method to examine the virus dilution end point.

**Yamaguchi K (1976). Resistance of the fall worm, *Hyphantria cunea*, to the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 45:377-378. [Japanese]**

**Watanabe H and Takamiya (1976). Susceptibility of the silkworm larvae, *Bombyx mori*, reared under different light conditions to polyhedrosis viruses. *J. Sericult. Sci. Jpn.* 45:403-406.**

In the present study, the effect of light rearing conditions on the susceptibility of the silkworm larvae to the polyhedrosis viruses was investigated. The susceptibility of larvae reared continuously in the dark to a nuclear- and cytoplasmic-polyhedrosis virus inoculated perorally was much higher than that of larvae reared continuously in the light. This effect of light rearing conditions on the susceptibility to viruses was more conspicuously observed in larvae reared on fresh mulberry leaves than in those fed on artificial diet. Histological observations of the midgets from larvae reared under different light conditions suggested that different physiology of midgut was directly induced by the effect of light conditions of larval rearing and thus made it possible to change the susceptibility of the silkworm larvae to viruses.

**Asayama T (1976). Electron microscope observation on the morphogenesis of the two strains of a nucleopolyhedrosis virus of the oak silkworm, *Antheraea pernyi*. *J. Sericult. Sci. Jpn.* 45:484-490.**

Comparative observation on the morphogenesis of a NPV of *Antheraea pernyi* was carried out between the strain forming multi-shaped inclusion body (MSIB) and the strain forming triangular inclusion body (TIB). Frequency distribution of the numbers of nucleocapsids observed in an envelope differed in the two virus strains. MSIB-NPV ranged from one to 19 with highest frequency at 4 nucleocapsids. On the other hand, TIB-NPV ranged from one to 12, and about 76% of the virus bundles consisted of one to 3 nucleocapsids. Size of nucleocapsid of the former was about 40 x 260-300 nm and the latter was about 40 x nm. MSIB-NPV was similar to the typical NPVs in the deposition process of inclusion body protein. On the contrary, adhesion of clumpy inclusion body protein at the surface of developing inclusion body was observed in

TIB-NPV. Formation of thick superficial layer in large inclusion body of TIB-NPV was observed and virion was not included in this zone. Membraneous structure closely associated with aligned nucleocapsids was also observed in the infected nucleus. The structure did not seem to have derived from nuclear membrane nor to become the envelope of nucleocapsid. Budding of nucleocapsids was seen at the surface of nuclear membrane.

**Miyajima S (1976). Studies on the hemagglutination with cytoplasmic-polyhedrosis virus of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 45:491-497.**

Hemagglutination (HA) reaction with purified CPV of the silkworm was investigated by the use of some mammalian erythrocytes. The results obtained are: 1. Purified CPV and CPV-infected midgut homogenate hemaglutinated chicken erythrocytes, but healthy midgut homogenate did not. 2. When several conc. of a chicken erythrocyte suspension in saline were mixed with an equal amount of the virus suspension in 0.01 M PBS saline (pH 6.8_, 0.4-0.5% erythrocyte suspensions always gave the best results. 3. When 4, 25 and 35°C were used for the reaction temperature, the most suitable result was obtained at 4°C. 4. The stable pH range of the reaction was b/w 5.5 and 8.6 at 4°C in PBS and the minimum quantity of the virus detectable was 4-12µg/ml. 5. The CPV hemagglutinated chicken, sheep and mouse erythrocytes, and the chicken erythrocyte was the most sensitive among them. 6. The HA value of CPV decreased gradually when CPV was kept at 4°C for more than two days. 7. When CPV was treated with ether, Tween 80-ether, sodium desoxycholte, fluorocarbon (once), formalin or hot water (90°C, 10 min). UV (40 min.), the HA value was not affected. 8. Hemagglutination inhibition (HI) reaction was positive on PV.

## Vol. 46 1977

**Inoue H (1977). Effect of the metamorphosis of the silkworm, *Bombyx mori*, on the multiplication of a flacherie virus. *J. Sericult. Sci. Jpn.* 46:20-24.**

The paper deals with the multiplication of a flacherie virus in the midgut epithelium during the process of larval-pupal and pupal-adult transformation. When virus infected 5$^{th}$ instar larvae become a pupa, all of larval midgut cells were discharged into the midgut lumen. The multiplication of the virus in the pupal midgut epithelium as detected by the fluorescent antibody technique was quite few and this fact was the same in the adult one. In the midgut lumen of the moth, cells showing weak fluorescence were present, and presumed to have been derived from the larvae.

**Nagae T (1977). The pathogenicity of *Streptococcus* bacteria isolated from the silkworm reared on an artificial diet III. Antibiotic resistance of the isolated *Streptococcus* bacteria. *J. Sericult. Sci. Jpn.* 46:25-31.**

Investigation was made on antibiotic resistance of *Streptococcus* bacteria isolated from silkworm larvae which had been reared on an artificial diet containing chloramphenicol or leucomycin. The results obtained were as follows:

(1) The isolated strains were resistant to each antibiotic administered. Therefore, the protection of silkworm larvae against these strains by the two antibiotics decreased markedly. (2) Cross resistance between leucomycin and other macrolide antibiotics was found among the isolated strains. (3) Thiopeptin, penicillin and streptomycin suppressed the infection of the inoculated strain which was resistant against chloramphenicol and leucomycin.

**Furusawa T and Kawase S (1977). Effect of protein synthesis inhibitors on virus specific RNA synthesis in the silkworm, *Bombyx mori*, infected with cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn.* 46:167-167. [Japanese]**

**Yamaguchi K (1977). Regeneration of the midgut epithelial cells in the silkworm, *Bombyx mori*, infected with cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn.* 46:179-180. [Japanese]**

**Abe Y (1977). On the crystallographic forms of polyhedral bodies of cytoplasmic polyhedrosis viruses originated from *Philosamia cynthia ricini*, *Lymantria fumida fumida* and *Dendrolimus spectabilis*, formed in the larval mid-gut epithelium of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 46:291-296.**

Three kinds of polyhedral bodies of CPV originated from PRCP, LFPC and DSCP when inoculated to newly hatched 2nd instar silkworm larvae, the crystallographic forms of LFCP and DSCP in the silkworm larvae showed some irregular forms, *ie*, square planes of dodecahedron with cube changed to rectangular planes and certain plain of rombic dodecahedron changed to pentagonal or hexagonal plane. But the crytalographic form of original PRCP, pentagonal dodecahedron was never changed in the sw.

**Furuta Y (1977). Studies on the small flacherie virus of the silkworm, *Bombyx mori* L. I. Difference between SFV and IFV in infectivity and the sensitivity to formaldehyde and heat. *J. Sericult. Sci. Jpn.* 46:297-300. [Japanese]**

**Abe Y (1977). Susceptibility of larval tissues of the silkworm, *Bombyx mori* L. to the nuclear polyhedrosis virus. III. Histogenesis of fat bodies during the moulting and its inhibition by the virus. *J. Sericult. Sci. Jpn.* 46:301-305.**

In the healthy larvae, fat body cells multiplied by mitosis before the moulting and the basement membrane of the cells disappeared during the moulting. Thereafter each cell was liberated. After the moulting fat body newly from the free cells. These histological changes of the fat bodies were observed in each larval moulting. On the other hand, afore-going regeneration of the fat bodies was not observed in the infected larvae, even in case where the moulting occurred in the infected larvae. The development of newly formed fat body in the larvae treated with low temperature was inhibited by the infection of nuclear polyhedrosis virus.

**Inoue H (1977). Thermal therapy of virus disease of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 46:306-312.**

The thermal therapy (37°C for 24h) of larvae infected with IFV was most effectively achieved during the molt or within 12h after edcysis. The heat treatment could be shorted upto 6h if it was performed just after ecdysis. The larvae, which had been administrated with lethal dose of IFV just after hatching, did not succumb to the disease, but made cocoons when repeatedly treated at 37°C for 24h at each larval ecdysis (4 times).

The multiplication of small flacherie virus SFV was inhibited at 37°C. When the SFV infected larvae were reared at 27°c after infection, the amount of SFV antigen, which was estimated by a single radial immunodiffusion test, of the midgut increased with time, but reduced at larval ecdysis. In addition, when the larvae were transferred from 27°C to 37°C after ecdysis, the reduction of SFV antigen was continued.

The formation of cytoplasmic polyhedra (cp) was inhibited at 37°C. When the larvae infected with CPV were reared at 27°C, the CPV infected cells were discharged from the midgut epithelium at ecdysis and the newly regenerated cells appeared at its place.

**Maeda S, Watanabe H and Matsui M (1977). Purification of an Ina-isolate virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 46:313-317.**

The virus has been demonstrated to be quite similar in pathological and chemical natures to the densonucleosis virus of *Galleria mellonella* and animal parvoviruses. Serological studies with the antiserum of purified Ina-isolate virus revealed that Ina-isolate virus is serologically quite different from an infectious flacherie virus of the silkworm.

**Iizuka T, Koike S and Mizutani J (1977). Antibacterial substances in feces of silkworm larvae. II. Quantities of some phenolic acids in feces of larvae reared on various artificial diets. *J. Sericult. Sci. Jpn.* 46:325-330.**

Protocatechuric acid (PA) is a main phenolic acid, followed by *p*-hydroxybenzonic acid (HA) is observed in the feces of silkworm larvae reared on mulberry leaves. In artificial diet fed silkworm larvae, caffeic acid (CA) was observed apart from PA and HA in minor quantities.

**Yanagita T and Saijo S (1977). Studies on the formaldehyde resistance of *Aspergillus* fungi attacking the silkworm larvae. I. The oxidizing action of formaldehyde by *Aspergillus* spp. *J. Sericult. Sci. Jpn.* 46:347-352. [Japanese]**

**Furuta Y (1977). Studies on the small flacherie virus of the silkworm, *Bombyx mori* L. II. Some characteristics of virus strains. *J. Sericult. Sci. Jpn.* 46:353-358. [Japanese]**

**Nagae T (1977). The pathogenicity of *Streptococcus* bacteria isolated from the silkworm reared on an artificial diet. IV. Effect of the dietary composition on the pathogenicity of *Streptococcus faecalis*. *J. Sericult. Sci. Jpn.* 46:384-390.**

*Streptococcus faecalis* A-2 showed no pathogenic activity to silkworm larvae reared on mulberry leaves.

**Inoue H (1977). Effect of low temperature on the multiplication of a flacherie virus in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 46:453-454. [Japanese]**

## Vol. 47 1978

**Kang SK, Nakagaki M, Shimizu T and Kawase S (1978). Purification of Ina-flacerie virus and some properties of its nucleic acid. *J. Sericult. Sci. Jpn.* 47:39-46.**

Ina-flacherie virus, which had been isolated in 1968 from flacherie diseased silkworm larvae in sericultural farms at Ina-shi, Nagano Prefecture, was purified and some properties of its nucleic acid were investigated. The results showed that the virus was a spherical particle with a diameter of about 20 nm, having the sedimentation coefficient of 100 s. Bothe fibrous and powdery nucleic acids were extracted from the purified virion and both were positive to diphenylamine reaction.

Evidence from formaldehyde reaction and staining properties with acridine orange indicated that the virus genome DNA was single stranded in intact virion, which resembled to the denso-nucleosis virus and adeno-associated virus. However, the fibrous nucleic acid extracted was double stranded. Electrophoresis of the virus DNA on polyacrylamide gel showed two bands. The results may indicate that this virus contains single complementary strands in separate virions and most parts of the nucleic acid become double stranded on extraction. Therefore wer consider that this virus belongs to *parvovirus* group.

**Kang SK and Kawase S (1978). Some properties of capsid protein of Ina-flacherie virus. *J. Sericult. Sci. Jpn.* 47:53-56.**

Purified preparations of Ina-flacherie virus protein were fractionated by polyacrylamide gel electrophoresis and amino acid composition was determined by amino acid analyser. Two polypeptide components, IP I and IP II were detected and the molecular weights of these compounds were 55,500 and 45,500 respectively. The IP II was major polypeptide comprised about 80% of the total virus protein.

Seventeen amino acids were detected by an amino acid analyzer from the hydrolyzate of the virus protein and the pattern of amino acid composition was similar to those of several other insect viruses.

**Abe Y (1978). Susceptibility of larval tissues in the silkworm, *Bombyx mroi* L., to the nuclear polyhedrosis virus. IV. Effects of virus infection on larval cuticle formation. *J. Sericult. Sci. Jpn.* 47:113-118.**

Histological observations were carried out on the ingegument of silkworm larvae infected with nuclear polyhedrosis virus at various stages of the 3rd instar to determine the effect of virus infection on the formation of new cuticle during the moulting. In healthy larvae the epidermal cell layer became thick by elongation of cells and glycogen was stored in the cytoplasm near the basement membrane before the 3rd moulting (60 h after the 2nd moulting). The larvae exuviated at 84 h after the 2nd moulting.

In larvae infected with the virus immediately after the 2nd moulting cytopathological changes caused by the virus were seen in epidermal cells at 36 h after the infection and cell layer did not become thick. Glycogen was not stored in the cytoplasm. The larvae died without forming the new cuticle. In larvae infected with the virus at 24 h after the 2nd moulting, cytopathological changes caused by the virus were observed in epidermal cells at 48 h after the infection and the cell layer became thick at 72 h after the 2nd moulting. But when most epidermal cells were infected, the storage of glycogen in the epidermal cells was not observed and no cuticle formation was observed. On the other hand, when the infected cells were very few, the storage of glycogen in the epidermal cells was observed and new cuticle was normally formed at 84 h after the 2nd moulting. In larvae infected with the virus at 36 h after the 2nd moulting, the epidermal cell layer became thick and glycogen was stored in the cytoplasm. The larvae could exuviate to next instar. The pathological changes of infected epidermal cells was observed 60 h after the inoculation.

**Sato F, Inoue H, Furuta Y and Ayzawa C (1978). Immunofluorescence observation on the multiplication of a small flacherie virus in the silkworm, *Bombyx mroi*. *J. Sericult. Sci. Jpn.* 47:171-174.**

In the present work, histopathological observations were carried out with the fluorescent antibody technique on the multiplication of a small flacherie virus in the silkworm. The frozen sections of the virus infected larvae showed that the virus specifically infected to the columnar cell of the midgut epithelium but scarecely multiplied in the other tissues such as foregut, hindgut, integument, silk gland, muscles, tracheal cells, Malpighian tubules, fat body and hemocytes. In the columnar cell, the virus formed virus matrix in the nucleus and the cell having giant nucleus reached up to 10 per cent of the virus infected cells at an advanced stage of infection.

**Sato F, Shimizu T and Inoue H (1978). Immunofluorescence observation on the formation of SFV antigen in the silkworm, *Bombyx mroi*, infected with an Ina-isolate virus. *J. Sericult. Sci. Jpn.* 47:175-176. [Japanese]**

**Watanabe H and Maeda S (1978). Genetic resistance to peroral infection with a densonucleosis virus in the silkworm, *Bombyx mroi*. *J. Sericult. Sci. Jpn.* 47:209-214.**

One of the most resistant and nonsusceptible strains to peroral infection with a *Bombyx* densonucleosis virs (DNV) was C124, while N124 strain was highly susceptible. The mode of inheritance of resistance as shown in C124 was investigated with individuals of the F1 and F2 and backcrossed hybrids obtained from crosses between the two inbred strains, C124 and N124. The results of the test revealed that susceptibilities of the reciprocal F1 hybrids of C124×N124 and the backcrossed hybrids to N124 were nearly the same as that of N124, whereas the F2 and the backcrossed hybrids to C124 showed much lower susceptibilities than N124. Comparative analysis of the log virus dosage percent infection (probit) lines among those hybrids indicated that the resistance in C124 strain was inherited as being controlled by a recessive major-gene.

**Abe Y (1978). Alteration of discharged *Nosema bombycis* sporoplasm in silkworm alimentary canal. *J. Sericult. Sci. Jpn.* 47:221-225.**

The spores treated with KOH solution or ingested by silkworm larvae changed their stainability. Namely inner part of spore lost its affinity for basic stain and two granules stained with acid dye were recognized. Small sporoplasms with poor cytoplasm, which had been discharged from spores, developed to large ovoid cells with two nuclei and one vacuole in the cytoplasm. They were observed in peritrophic membrane, so that they seemed to be protected by the membrane. Some sporoplasm were observed in the area of microvilli.

**Furuta Y (1978). On the heredity of the resistance to small flacherie virus in the silkworm, *Bombyx mroi*. *J. Sericult. Sci. Jpn.* 47:241-242. [Japanese]**

**Abe Y (1978). Propagative reproduction of *Nosema bombycis* NAGEGELI in silkworm larvae of the silkworm. *J. Sericult. Sci. Jpn.* 47:279-284.**

Histological observation of infected silkworm larvae, which were perorally administered spores of *Nosema bombycis* in the 2$^{nd}$ instar revealed that the *Noseme* cells occurred only in granulocytes in 24 h. The parasite cells developed to round acidophilic cells (APC) in vesicles of the granulocytes, some of them were released into the blood stream and entered the muscle layer of midgut in 48 h. Cytoplasms of midgut epithelial cells, silk gland cells, and Malpighian tubule cells were found to contain APC and empty vesicles at the distal end near the basement membrane and young and matured sporoblasts at the proximal end near the lumen in 72 h. The sporoblasts had developed to the spores in 96 h. At this time, large parasite cells appeared in granulocyte and were liberated into the hemocoel. The cells became amoeboid cells with two nuclei and invaded other host cells. APC appeared to be responsible for earlier propagation, while amoeboid cells engage in later spread.

**Abe Y (1978). The life cycle of *Leptomonas* sp., a protozoan parasite of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 47:421-426.**

**Kobayashi M, Nakagaki M and Kawase S (1978). Chromatic Droplets during Histolysis of Midgut of the silkworm, *Bombyx mroi*. *J. Sericult. Sci. Jpn.* 47:440-444. [English]**

In the present paper, we examined histochemically the pupal midgut and found that the midgut contents contained a lot of granules which gave strong Feulgen reaction. These granules suggested that they corresponded to the `chromatic droplets' which had been observed in insect tissues during larval molting and metamorphosis. The emphasis of this work is put on that the formation of these granules may be attributable to conformational alterations of chromatin.

## Vol. 48 1979

**Noguchi Y and Yamaguchi K (1979). An immune response of the fall webworm, *Hyphantria cunea*, to the infection by a cytoplasmic-polyhedrosis virus of the silkworm, *Bombyx mroi*. *J. Sericult. Sci. Jpn.* 48:15-18.**

Although the larvae of fall webworms, *Hyphantria cunea*, were susceptible to the infection by a cytoplasmic polyhedrosis virus (CPV) of the silkworm, *Bombyx mori*, almost all of the infected larvae recover from the virus infection. The natural cure was apparently due to the larval capacity to replace the infected midgut cells by the renewed epithelial cells at the molt. The fall webworm larvae thus being naturally cured showed the marked resistance to the infection by a silkworm CPV administered seconderly whereas the fresh control larvae were quite susceptible. The mechanism of immune response of the larvae to the virus infection was discussed.

**Abe Y and Fujiwara T (1979). Mode of multiplication of a protozoan, *Pleistophora* sp. (Microsporidia: Nosimatidae), in the midgut epithelium of silkworm larvae. *J. Sericult. Sci. Jpn.* 48:19-23.**

Histological observations showed that *Pleistophora* sp. multiplied by nuclear division in the cytoplasm of cylindrical cells of anterior portion of the midgut of silkworm larvae, when this microorganism was administered perorally. The parasite first became multinucleate baton-like in these cells and then divided into binucleate round forms. These forms passed into the midgut lumen and invaded into another cylindrical cells.

Inoculated protozoan also parasitized posterior portion of the midgut. In this case, however, the binucleate round forms originating from multinucleate baton like forms in the cylindrical cells did not pass to the lumen but transformed into pansporoblasts and later to mature spores in the same host cells.

**Iwamoto A and Eguchi M (1979). Comparison of three proteases in the digestive juice of the silkworm: Substrate specificity and the effect of inhibitors. *J. Sericult. Sci. Jpn.* 48:31-36 [Japanese]**

**Kawakami K and Naka A (1979). Detection of an entomogenous fungus, *Metarhizium anisopliae* from the soil of mulberry fields and their pathogenicity to the silkworm. *J. Sericult. Sci. Jpn.* 48:43-52.**

An entomogenous fungus, *Metarhizium anisopliae* (METSCHNIKOFF) SOROKIN is well known as the pathogen of “green muscardine” in the muscardine diseases of the silkworm. *Bombyx mori* and other wild insects. It has beenn believed that the main source of the fungus infection of the silkworm are wild insects attacked by muscardine fungi spontaneously. Therefore, in order to improve the control methods of silkworm muscardine, it is necessary to know whether thre are any other sources of the fungus infection to the silkworm in fields. The present paper deals with *Metarhizium anisopliae* distributed in the soild of mulberry fields as the primary source of the fungus infection to the silkworm.

An entomopathogenic fungus, *Metarhizium anisopliae* was easily detected in the mulberry field soild by the dilution plate method using 0.02% Tween 40 sterile solution and the selective agar medium. Czapek Dox-rose Bengal (60 mg/l) chloramphenicol (0.1mg/ml) cyclohexamide (1.0 mg/ml) agar (CRC agar) or by the baiting (trapping) method using living silkworm pupae. Many isolates of *M. anisopliae* from the soil of the mulberry fields showed high pathogenicity to silkworm larvae. Isolates of *M. anisopliae* obtained from the mulberry field soil were divided into three forms into three forms in their conidial size and in colour of colony: a short spored form with greenish black colony (the most common form, *M. Anisopliae* (METSCHNIKOFF)

SOROKIN var. *anisopliae)*, a short spored form with pale green colony (*M. anisopliae* var. *anisopliae*) an a long spored form with greenish black colony (*M. anisopliae* (METSCHNIKOFF) SOROKIN var. *major* TULLOCH). Foregoing results suggest that *M. anisopliae* is distributed in the soil as a soil born fungus not only over winter in the soil of mulberry fields as reservoir, but also grow in the soil.

**Eguchi M, Iwamoto A and Yamaguchi K (1979). Relationship among protease from the midgut, peritrophic membrane and digestive fluid of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 48:53-58.**

The relationship among protease of the midgut tissue, peritrophic membrane and digestive fluid was investigated. In the elution pattern from Sepharose 6B of these proteases, the first peak of midgut protease which is considered to be a membrane bound enzyme accorded with that of the peritrophic membrane. The similarity in elution positions of free enzymes (second and third peaks) was seen among the midgut, peritrophic membrane and digestive fluid. The enzyme corresponding to the second peak of digestive fluild protease was produced by the solubilisation of midgut protease with Lubrol WX. The protease activity of the peritrophic membrane per wet weight was low and comparable to that of the midgut, whereas the protease showed high specific activity (activity per mg of protein) which was little lower than that of the digestive fluid. The experiment by Ouchterlony's double diffusion method revealed that protease of digestive fluid and peritrophic membrane and one of midgut proteases are identical immunologically. The result obtained suggest that proteases transported from the midgut epithelium are reversed and concentrated in the peritrophic membrane and discharged into the lumen as free enzymes.

**Eguchi M, Haneda I and Iwamoto A (1979). Protease inhibitors in the haemolymph of the silkworm, *Bombyx mori*: their effect on the protease from the digestive fluid and the midgut of the silkworm. *J. Sericult. Sci. Jpn.* 48:89-95.**

The effect of inhibitors in the haemolymph of the silkworm on the proteases from the digestive fluid and midgut of the silkworm was studied and compared with their effect on trypsin and chymotrypsin. Protease activity of the midgut and the digestive fluid was inhibited by the addition of haemolymph. The enzyme activity-haemolymph concentration curve of digestive fluid protease was similar to that of trypsin, but curve of midgut protease was somewhat different from the above proteases. Three inhibitors were demonstrated by the gel filtration of haemolymph of Sephadex G-75, the first fraction inhibited the digestive fluid and midgut proteases, the third one was effective to trypsin and chymotrypsin and the second one inhibited all the four proteases. The first and third fractions were heat stable but the second one was heat labile. The inhibitory effect decreased during the fifth instar, increased just before pupation and there was no great change in the pupal stage.

**Iizuka T, Koike S and Mizutani J (1979). Antibacterial activity of some low molecular substances in the digestive juice of silkworm larvae, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 48:96-100.**

A main antibacterial activity in the digestive juice of silkworm larvae fed on mulberry leaves was investigated by using dialyzed digestive juice and ethyl acetate soluble fractions of digestive juice. In the above experiments, it was recognized that some low molecular substances in the digestive juice had a main antibacterial activity.

As low molecular antibacterial substances from the digestive juice of silkworm larvae, caffeic acid (CA) and protocatechuic acid (PA) were isolated by Koike *et al* (1978) and D(-)-2,3-diaminopropionic acid which Wada and Toyota (1965) already isolated from the digestive juice was recognized as antibacterial substance by Iizuka (1977). In these experiments the antibacterial

activities of CA, PA, quinic acid and chlorogenic acid (ChA) were investigated in the synthetic medium adjusted to pH 7.0 and pH 10.0 respectively.

As a result of these experiments, in the synthetic medium at pH 10.0, 500 ppm of CA shows evidently antibacterial activity, which ChA does not show the activity even in the medium containing 1000 ppm. However, when 1000 ppm of ChA was added to the medium containing 500 ppm of CA, the antibacterial activity was extremely increased. This synergistic action must play an important part in the control of bacterial flora in the gut of silkworm larvae.

**Abe Y (1979). Anti-trypanosomal effect of acridine orange, sodium azide or high temperature on *Letomonas* sp. (Protomonadina; Trypanpsomatidae). *J. Sericult. Sci. Jpn.* 48:106-110.**

Anti-trypanosomal action of acridine orange, sodium azide and high temperatures on *Leptomonas* sp. cultured *in vitro* and in the pupal hemocoel of the silkworm, *Bombyx mori* was investigated. Acridine orange and sodium azide stopped the multiplication of the flagellates cultured *in vitro*, but did not so *in vivo*. On the contrary, the flagellate, when exposed to temperatures higher than 35°C for 24 h died both *in vivo* and *in vitro*. Theflagellate appeared to be eliminated from the hemolymph by phagocytic action of the host insect since degenerated protozoans were detected in the blood cells.

**Iwano H and Ishihara R (1979). Temperature and effects of chemical stimuli to hatch of *Nosema bombycis* spores. *J. Sericult. Sci. Jpn.* 48:142-146.**

It is known that *Nosema bombycis* spores are stimulated to hatch by either $H_2O_2$+KCl mixture or 0.1 M K2CO3+KHCO3 mixture (pH 10.7) at 25°C. Hatching also depends on the medium temperature as was already reported, when primed by pH shift from high to neutral and by Sorensen buffer with KCl as well.

Attempts were made to differentiate the steps of *N. bombycis* spore hatch. Spores, when exposed to $H_2O_2$ at 5°C, were primed to hatch in distilled water at 25°C after thoroughly washed with distilled water to remove $H_2O_2$. Spores, however, did not hatch in distilled water at 25°C after suspended in 0.1 M $K_2CO_3$+$KHCO_3$ mixture (pH 10.7) at 5°C. Under 40°C on the other hand, suspended in $H_2O_2$+KCl mixture hatched, but did not so in $K_2CO_3$+$KHCO_3$ mixture. Those spores suspended in the latter medium, however, were partially primed to hatch in distilled water at 25°C. These results indicate that the mode of action of these two mixtures on the hatch of *N. bombycis* spores is different. Besides there are at least two steps in hatch, that is, the step for priming by chemicals followed by another step requiring temperature only.

**Abe Y and Higuchi Y (1979). Histological observation of the integument in larvae of *Antheraea yamamai*, infected with a nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 48:179-180. [Japanese]**

**Sakamoto E and Horie Y (1979). Quantitative Change of Phosphorus Compounds in Hemolymph during Development of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 48:319-326. [English]**

Phosphorus compound in haemolymph of the silkworm, *Bombyx mori* were determined during larval and pupal stages. The results are summarized as follows:

(1) The total amounts of phosphates in hemolymph ranged from 30 to 80 μ moles per ml and elevated in the latter half of the $5^{th}$ instar. The total phosphates were large in amount in male than those in female in pupal stage. (2) Acid soluble phosphate comprised a large portion of the total phosphate during larval and pupal stages whereas content of inorganic phosphate was

approximately 10% of total phosphate. (3) The presence of large amount of S-6-P and little amount of both G-6-P and inorganic phosphate were detected in hemolymph of the 5th instar larvae by liquid chromatography, whereas S-6-P and G-6-P were scarcely found in hemolymph of pupae. (4) The quantitative changes of polyol phosphate and trehalose in hemolymph were determined.

In the 5th instar larvae hemolymph S-6-P ranged from 13 to 23 μ moles per ml, being approximately twice as much as that of trehalose. The metabolic significance of S-6-P in the silkworm was briefly discussed.

**Kunimi Y and Morita Y (1979). Effect of the larval stage and the food condition on the susceptibility of the silkworm, *Bombyx mori*, to a cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 48:332-336.**

Changes in the susceptibility of the silkworm, *Bombyx mori* to a cytoplasmic polyhedrosis virus (CPV) during the 3rd instar were investigated using larvae in three different food conditions: rearing with mulberry leaves (ML-rearing), rearing with artificial diet (AD-rearing) and rearing with artificial diet from the first to second instar and with mulberry leaves thereafter (AM-rearing). The susceptibility of larvae to CPV in each rearing was high in the early stage, especially immediately after the second moult and was reduced until the middle stage and then rapidly increased up to the 3rd moult. The larvae in AM-rearing immediately after the 2nd moult were more susceptible than those in ML-rearing. However, at 10, 24 and 48 h after the first feeding of the 3rd instar, the larvae in AM rearing were less susceptible than those in ML-rearing.

**Fujiwara T (1979). Infectivity and pathogenicity of *Nosema bombycis* to larvae of the silkworm. *J. Sericult. Sci. Jpn.* 48:376-380. [Japanese]**

**Furuta Y (1979). Effects of physiochemical treatment on the antigenicity and pathogenicity of flacherie virus in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 48:444-448. [Japanese]**

**Kato Y, Nakayama S and Takeuchi T (1979). Fractionation of PROPERTIES OF Fraction III Protein in the Haemolymph of the Silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 48:484-490. [English]**

Fraction III protein, fractionated from haemolymph protein of silkworm larvae by gel filtration on Sephadex G-150 column, was separated into three peaks, III-A, III-B and III-C, by ion exchange chromatography on DEAE-celluloe column. Each fraction was further fractionated into two or three peaks on a TEAE cellulose column. These peaks separated from each of three fractions, III-A, III-B and III-C were termed III-$A_1$, III-$A_2$, III-$A_3$, III-$B_1$, III-$B_2$, III-$C_1$, III-$C_2$ and III-$C_3$. Each of the components was examined by polyacrylamide gel electrophoresis and all were heterogeneous. The estimation of molecular weights (MW) of the eight components was performed by SDS-polyacrylamide gel electrophoresis. III-$A_1$ gave two bands (MW 23,000 and 74,000). III-$A_2$ and III-$A_3$ age a single band corresponding to MW 28,000 and 24,000 respectively. III-$C_1$ gave a single band (MW 25,000), III-$C_2$ four bands (the lowest MW 26,000; the highest MW 64,000) and III-C3 also showed four bands (the lowest MW 26,000; the highest MW 64,000).

## Vol. 49 1980

**Yanagita T (1980). Studies on the formaldehyde resistance of *Aspergillus* fungi attacking the silkworm larvae. II. Aldehyde dehydrogenase of *Aspergillus* spp. *J. Sericult. Sci. Jpn.* 49:45-50.**

In the process of investigation on the resistance of *Aspergillus* fungi to formaldehyde, the presence of an aldehyde dehydrogenase in the fungus cells was discovered. The enzyme was partially purified from the mycelial mats *Aspergillus flavus-oryzae* by precipitating with ammonium sulphate and acetone. Enzyme activity was greatly accelerated by the addition of glutathione. The optimum pH for the oxidation of formaldehyde by this enzyme was determined to be 8.1. The enzyme oxidized formaldehyde and acetaldehyde and the activity for formaldehyde was much higher than that for acetaldehyde. Other aldehydes and alcohols were not oxidized by this enzyme. This enzyme was unstable at room temperatures (22-25°).

**Yanagita T (1980). Studies on the formaldehyde resistance of *Aspergillus* fungi attacking the silkworm larvae. III. Relation between the formldehyde resistance and the aldehyde dehydrogenase activity of *Aspergillus* spp. *J. Sericult. Sci. Jpn.* 49:51-56.**

Cultured *Aspergillus* fungi showed the maximum aldehyde dehydrogensase activity in 7 days at 30°C. The enzyme activity was detected in isolates of *Aspergillus flavus-oryzae* group, *A. tamari* group and *A. ochraceus* group. It was also observed in *Aspergillus oryzae* which had never been in contact with formaldehyde.

However, the activity differed between species of *Aspergillus* according to their formaldehyde resistance: the resistant fungus isolate showed high enzyme activity and vice versa. The enzyme activity was increased by subculture on a medium containing formaldehyde.

**Miyagawa M and Sakai H (1980). On a previously unreported strain of the cytoplasmic-polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 49:169-170. [Japanese]**

**Tanaka S and Shimizu T (1980). Spread of a nuclear polyhedrosis in silkworm rearing on artificial diet. *J. Sericult. Sci. Jpn.* 49:211-217. [Japanese]**

**Kagawa T (1980). The efficacy of formalin as disinfectant of *Nosema bombycis* spores. *J. Sericult. Sci. Jpn.* 49:218-222.**

The present study was carried out to clarifying the relationships between the conditions of formalin treatment (concentration, length of the treatment and temperature) and the death rate of *Nosema bombycis* spores.

With the increase of concentration, the extension of period or rise of temperature, the death rate of spores increased. These factors when combined intensify the action of formalin on spores. The efficacy of formalin as disinfectant for *Nosema bombycis* spores under a certain temperature, relates to the combination of its concentration and the length of treatment, that is, the critical range of effective disinfection can be expressed in terms of the product of value of the formalin concentration and the length of the treatment.

**Fujiwara T (1980). Three microsporidians (*Nosema* spp.) from the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 49:229-236.**

Three microsporidians belonging to the genus *Nosema* were found in silkworm adults which had been raised by the silkworm growers in Japan and tentatively designated as M11, M12 and M14. This paper deals with their biological characteristics and their pathological effects to silkworm larvae. *Nosema* sp. (M11): Site of infection: malpighian tubule, silkgland, muscale and fat body; Sporulation stages: a sporont divides into two sporoblasts; sporont size 4~6 × 3~4 μm, sporoblast 4~6 × 2~4 μm, spore: ovocylindrical; 3.9 × 1.7 μm; polar filament 83 μm (79~90 μm) in length. Locality: Ibaraki-ken; Pathogenicity: lower than *N. bombycis*.

*Nosema* sp. (M12): Site of infection: malpighian tubule, silkgland, muscale and fat body with hypertrophy; Sporulation stage: a sporont divides into two sporoblasts; sporont size 5~7 × 3~5 μm, sporoblast 7~8 × 3~4 μm, spore: ovocylindrical; 5.1 × 2.1 μm; polar filament 118 μm (106~133 μm) in length. Locality: Chiba-ken; Pathogenicity: as high as *N. bombycis*.

*Nosema* sp. (M14): Site of infection: malpighian tubule, silkgland, muscale and fat body; Sporulation stages: a sporont divides into two sporoblasts; sporont size 5~7 × 3~5 μm, sporoblast 4~7 × 3~5 μm, spore: ovoidal; 4.2 × 2.4 μm; polar filament 106 μm (106~115 μm) in length. Locality: Niigata-ken; Pathogenicity: lower than *N. bombycis*.

**Tanaka S, Hara M and Ishisaka T (1980). The relationship between the incidence of nuclear polyhedrosis in silkworm larvae reared with an artificial diet during the young stage and the rearing period in the Sericultural farms. *J. Sericult. Sci. Jpn.* 49:247-248. [Japanese]**

**Yanagita T (1980). Studies on the formaldehyde resistance of *Aspergillus* fungi attacking the silkworm larvae. IV. Relation between pathogenicity between pathogenicity to silkworm larvae and chitinase activity of *Aspergillus flavus-oryzae*. *J. Sericult. Sci. Jpn.* 49:440-445.**

Formaldehyde resistance of *Aspergillus* fungi was investigated by the analysis of pathogenicity against the silkworm in relation to enzymatic action in *Aspergillus flavus-oryzae* fungi. No relationship existed between the activity of lipase and cellulase and pathogenicity to silkworm larvae. The chitinolytic action of *Aspergillus flavus-oryzae* involved both liquefying and saccharifying activities and the rate of liquefying activity was higher than that of the saccharifying activity. The activity of chitinolytic enzyme, bothe liquefying and saccharifying, in the fungi was found to change parallel to pathogenicity against silkworm larvae.

**Yanagita T (1980). Studies on the formaldehyde resistance of *Aspergillus* fungi attacking the silkworm larvae. V. On the decomposition of chitin of larval skin by *Aspergillus flavus-oryzae* fungi. *J. Sericult. Sci. Jpn.* 49:446-450.**

The mechanism of the fungus invasion through the skin of silkworm larvae was investigated by determining the decomposition of chitin in the cuticle by both liquefying or saccharifying activities of the chitinolytic enzyme of *Aspergillus flavus-oryzae*. When the newly hatched larvae were inoculated with *Aspergillus flavus-oryzae*, amino sugars were easily extracted from the larvae and the polymerization degree of chitin decreased. Such a decomposition of chitin was marked at a early time in the growth of the fungi. The decomposition of chitin as determined by the liquefying activity of *Aspergillus flavus-oryzae* occurred in parallel with pathogenicity.

**Kawase S, Hashimoto Y and Nakagaki M (1980). Characterization of flacherie virus of silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 49:477-484.**

Flacherie viruses of the silkworm, *Bombyx mori* were purified and following results were obtained. Nucleic acid of flacherie virus (FV) of the silkworm was single stranded RNA, which had a molecular weight (MW) of $2.4\times10^6$ daltons and the RNA content in the virion was 28.5%. Four structural proteins VP1 (MW: 31,000-32,0000), VP2 (MW: 41,000-42000), VP3 (MW: 49,000) and VP4 (MW: 68,000-69,000) were found in the virion and the VP1 was major capsid protein accounting for 70% of the total virion proteins. These data suggesst that FV belongs to picornavirus group and the cryptogram of FV was determined to be R/1:2.4/29:S/S:I/O.

**Watanabe H and Shimizu T (1980). Epizootiological studies on the occurrence of densonucleosis in the silkworm, *Bombyx mori*, reared at sericultural farms. *J. Sericult. Sci. Jpn.* 49:485-492.**

Epizootiological investigations were made on the occurrence of densonucleosis at sericultural farms in 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 Prefectures. The enzootic was mainly due to the rearing of the silkworm strains of nonsusceptible or highly resistant to the infection of densonucleosis virus. However, densonucleosis virus was generally detected in the dust from every farm in Nano Pfefecture, even from farms where nonsusceptible silkworm strains were reared and no occurrence of densonucleosis had been seen. It was found that the virus detected in the dust was derived from contaminated mulberry leves with a densonucleosis virus which multiplied chronically in the mulberry pyralid, *Glyphodes pyloalis*, infesting the mulberry plantation. Under these circumstances, rearing of the nonsusceptible silkworm sgtrain seemed to be one of the best ways to protect the densonucleosis in the farm. In Saitama Prefecture, on the other hand the densonucleosis virus was not detected in the dust from any farm, nor densonucleosis infection in the mulberry pyralid was recognized.

**Sato R and Watanabe H (1980). Purification of mature microsporidian spores by iso-density equilibrium centrifugation. *J. Sericult. Sci. Jpn.* 49:512-516.**

The present study was made to establish the purification method of mature microsporidian spores by iso-density equilibrium technique using sucrose or Percoll (colloidal silica, Pharmacia). Although it was unsuccessful to isolate mature spores from any of immature spores, host tissue debrises and bacteria by centrifugation using buffered Percoll. After centrifugation of Percoll mixed with partially purified *Nosema bombycis* spores in Beckman Type 65 rotor at 78,000g for 30 min., a band consisting of mature spores was exclusively obtained. The recovery rate of mature spores averaged over 90% and no damage to the spores in the course of purification was observed.

## Vol. 50 1981

**Kato S (1981). Extraction of a pathogenic substance from *Hafnia* sp., a bacterial pathogen of flacherie of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 50:47-50. [Japanese]**

**Tanaka S and Shimizu T (1981). Spread of a nuclear polyhedrosis in silkworm rearing on artificial diet (Continued). *J. Sericult. Sci. Jpn.* 50:115-119. [Japanese]**

**Iizuka T, Faust RM and Travers RS (1981). Isolation and partial Characterisation of Extrachromosomal DNA from Serotypes of *Bacillus thuringiensis* Pathogenic to Lepidopteran and Dipteran Larvae by Agarose Gel Electrophoresis. *J. Sericult. Sci. Jpn.* 50:120-133. [English]**

Extrachromosomal DNA molecules of 24 different *Bacillus thuringiensis* strains were consistently isolated using a procedure which optimizes lysis of the normally lysozyme resistant vegetative cells. 24 strains of *Bacillus thuringiensis* contained extrachromosomal DNA molecules of various sizes that were readily visualized with agarose gel electrophoresis and that ranged from >200 to <1 megadaltons (Mdal), depending on the strain. A determination of the numbers of extra chromosomal DNA molecues based on agarose gel profiles revealed that there were 1 for *sotto* and *thompsoni*, 2 for *subtoxicus, entomocidins, ostriniae and dermatadiens*, 3 for *dendrolimus* and *Indiana*, 5 for *galleriae*, 6 for *canadermis*, *morrisoni* and *isralensis*, 7 for *wuhanensis*, 8 for *kenye, tolworthi* and *pakistani*, 9 for *thuringiensis* (BA 068) and *dakota*, 10 for *toumanoffi*, 11 for *thuringiensis*, 14 for for *kyushuensis* and 16 for *Kurstaki* (HD-1). Such abundance and variety of extrachromosomal DNA elements is a rare phenomenone among bacterial species. In serologically identical varieties (*Thuringiensis* Berliner and *thuringiensis* BA-068, serotype-1, *sotto* and *entomodicus*, serotype 6), only serotype 6 strains show similar extrachromosomal DNA profiles and while a number of strtains of *Bacillus thuringiensis* have

some extrachromasomal DNA elements in common, distinct differences can be observed in the DNA profiles between serotypes and even among strains of the same serotype.

**Watanabe H and Shimizu T (1981). Factors concerned with the epizootics of nuclear polyhedrosis in the silkworm observed recently in sericultural farms. *J. Sericult. Sci. Jpn.* 50:146-153.**

The epizootiological investigations on the occurrence of diseases in Sericultural farms in recent years suggested that the nuclear polyhedrosis has become dominated to occur throughout several prefectrures, especially in summer and fall rearing seasons. As factors concerned with epizootics of nuclear polyhedrosis, neither wide spread of a high virulent nuclear-polyhedrosis virus (NPV) in Sericultural farms, nor the rearing of sw strains with decreased resistance were recognized. On the other hand, incomplete disinfection that allows to remain some amont of viable viruses in the rearing house was frequently observed in the most of the Sericultural farms. Among sw viruses, NPV showed the highest viability under the natural condition within the rearing house. Accordingly, as far as incomplete disinfection of rearing house and instruments was carried out, it was highly possible that the recent epizootics of nuclear polyhedrosis much depends on the repeated infection with NPV survived from disinfection and the increasing infestation of NPV during the multiple silkworm rearings.

**Sato R, Kobayashi M, Watanabe H and Fujiwara T (1981). Serological discrimination of several kinds of microsporidian spores isolated from the silkworm, *Bombyx mori*, by an indirect fluorescent antibody technique. *J. Sericult. Sci. Jpn.* 50:180-184.**

*Nosema bombycis* and seven kinds of microsporidia isolated from the infected silkworm were used in the present study. These microsporidia were different in the pathogenicity, the target tissue and the spore morphology. However, indirect fluorescent antibody tests of these Microsporidian spores revealed that the four of them were serologically the same with *N. bombycis*, suggesting they belonged to the common species. On the contrary, the other three Microsporidian spores reacted only with each homologous antiserum. These results indicated that the indirect fluorescent antibody test of spores seemed to be one of the most useful methods for species discrimination of microsporidia being infectious to the sw.

**Matsubara F, Ohnishi M, Matsumoto T and Hayashiya K (1981). Studies on the application of rearing of the silkworm with aseptic method to sericulture. I. Groping for stage to transfer the larvae fed on artificial diet from aseptic to natural rearings from the view point of the resistivity to virus infection. *J. Sericult. Sci. Jpn.* 50:259-265.**

This report is concerning to grope the proper stage to switch over the artificial diet to mulberry leaves during larval raising period from the view point of susceptibility of the larvae to virus. About 200 thousands of sw larvae were rearing on artificial diet in the bioclean roon. Four groups of larvae were set and reared on artificial diet under aseptic state, during 1) $1^{st}$ stage only, 2) $1^{st}$ and $2^{nd}$ stages, 3) $1^{st}$ to $3^{rd}$ stages, 4) $1^{st}$ to $4^{th}$ stages, respectively and then reared naturally on mulberry leaves to final stage. Susceptibilities to virus infection were tested by means of A) cold treatment at 5°C for induction, B) oral administration and C) subcutaneous injection of NPV respectively.

Occurrence of the disease in the aseptic larvae fed on an artificial diet during early developmental stage, from the group 2) to the group 4), was lower than that in the larvae fed on mulberry for whole stages. In the case of A), each larval group reared on artificial diet from the $1^{st}$ to $2^{nd}$, to the $3^{rd}$ or to the $4^{th}$ instars showed lower induction rate in the order than the larvae reared naturally on mulberry leaves. In case of B) and C), however, the susceptibilities to NPV administrated increased in the decreasing order of raising period on mulberry leaves.

It was concluded from these data that the stage to transfer the larvae fed aseptically on artificial diet to natural stage should be 3d or 4th instar.

**Matsubara F, Ohnishi M, Matsumoto T and Hayashiya K (1981). Studies on the application of aseptic rearing method of silkworm to sericulture. II. Effect of multiple rearing on the susceptibility of virus infection of the silkworm larvae fed on artificial diet for younger stages. *J. Sericult. Sci. Jpn.* 50:266-270.**

Susceptibilities to virus infection of the silkworm larvae which had been reared aseptically on an artificial diet for younger stages and then transferred to rear on the mulberry leaves wee examined. This investigation were carried out six rearing times in a year.

1. Virus susceptibilities of the larvae to oral administration and subcutaneous injection with a nucear polyhedrosis virus (NPV) did not vary through rearing seasons of a year.
2. The occurrence of disease by administration of a cytoplasmic polyhedrosis virus (CPV) was low.
3. The larvae which had been fed on artificial diet in the bioclean room for younger stages grew normally after they were transferred to the mulberry leaf feading without any effect of seasonal variation in the quality of mulberry leaves.

**Iwano H and Ishihara R (1981). Inhibitory effect of several chemicals against the hatch of *Nosema bombycis* spores. *J. Sericult. Sci. Jpn.* 50:276-281.**

Of nine kinds of chemicals tested, 0.001% solution of Hilite® (Potassium Dichloroisocyanurate) inhibited, immediately, after treatment, the hatch of *N. bombycis* spores most effectively, followed by 0.1% of Benzethonium Chloride, Cocktone® and One Stroke Embiron®. These chemicals hold their inhibitory effect on spores at room temperature, for 24h after preparation.

Feeding test of *N. bombycis* spores with mulberry leaves showed spres treated with Hilite® lost their infectivity to the sw. Hilite® did not retarded the growth of sw larvae when fed with leaves at the concentration of 0.1%.

**Hukuhara T, Midirikawa M and Ito H (1981). Purification of a cytoplasmic-polyhedrosis virus from silkworm feces. *J. Sericult. Sci. Jpn.* 50:301-305.**

Groups of 5th instar larvae were administered an artificial diet wich had been contaminated with polyhedra of a cytoplasmic polyhedrosis virus. After feeding for two or three days on this diet, the larvae were transferred to uncontaminated diet and their feces were daily collected. The feces (1g) were macerated in 10 ml of 0.05-0.1 M sodium pyrophosphate to detach the adsorbing virus and incorporated into an aqueous two phase separation system consisting of 5 ml of 20% (w/w) dextran (MW 200,000-300,000) and 2 ml of 40% (w/w) polyethylene glycol 6000. The detached virus entered the upper phase, while the larger particles constituting the feces entered the lower phase. The upper phase was transferred to another vial and 1/100 volume of 20% (w/w) detran sulfate 500 (MW 500,000) and 1/10 volume of 3 M NaCl were added. In this biphasic system the virus was concentrated in the lower detran sulfate-rich phase. Serial dilutions of the macerated feces and the purified preparation were perorally administered to newly-hatched larvae in order to determine their $IC_{50}$ as expressed by dry weight per unit volume. The $IC_{50}$ of the starting material was 158 times as great as that of the purified preparation. This result indicated that most (157/158) of the non-viral fecal material was eliminated by the purification procedure. Feces collected on different days were compared in their infectivity by bioassays of the preparations purified from them. The feces defecated during the period when the larvae were fed virus-contaminated diet were highly infective and presumed to contain ingested polyhedra

and virions liberated from them. The feces collected 1-2 days after the larvae were transferred to uncontaminated diet showed very low infectivity. However, the infectivity began to increase in 4 to 5 days following the peroral administration of polyhedra and reached a very high level on the last day of the fifth instar. These feces were presumed to contain the virus which had multiplied in the epithelial cells of the larval midgut.

**Inoue H (1981). Double infection of midgut epithelial cells with nuclear and cytoplasmic-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 50:311-319.**

Double infection of a nuclear polyhedrosis virus (NPV) and a cytoplasmic polyhedrosis virus (CPV) were investigated histopathologically on the midgut in the sw, *Bombyx mori*. Fifth instar larvae were subjected to low temperature (5°C) for 24 h after ecdysis and then administered with a mixed suspension of NPV and CPV, which formed a big CPV-inclusion body in the nucleus of the columnar cell, and reared at 23°C. Double infection occurred in not a few columnar cells. Proteinaceous crystals of CPV and NPV were distinguishable by (1) stainability to bromophenol blue after pretreatment with methanol and hot water, CPV-inclusion body was stained but not NPV-polyhedron, (2) size and (3) crystal form. Both viruses were observed as being independent multiplication and fusion of two kinds of proteinaceous crystals was not occurred; however, lots of numbers of nucleocapcid of NPV attached to the surface of CPV-inclusion body, followed with incorporation of some of them into the inclusion body, and othat was in contrast to NPV-polyhedron incorporating enveloped NPV and its empty envelops.

**Hukuhara T and Midorikawa M (1981). Assessment of the occurrence of the cytoplasmic polyhedrosis in silkworm population reared on an artificial diet. *J. Sericult. Sci. Jpn.* 50:345-346. [Japanese]**

**Shimizu T and Komori S (1981). Pathogens from cocoons soiled inside and their tolerance to dry heat treatment. *J. Sericult. Sci. Jpn.* 50:355-358.**

It sometimes happens that the inner surface of a cocoon is soiled by the injured or diseased prepupa or pupa in it. The authors examined such sort of cocoons pathologically.

(1) As high as 70.45% out of 952 cocoons, previously dried in the filature factories, were fond to be contaminated with nuclear polyhedrosis. However, a lower rate of contamination was found from such sort of raw cocoons from farmers. (2) The nuclear polyhedra and the toxic crystals of *Bacillus thuringiensis* were not inactivated by the routine treatment during cocoon-drying before silk reeling. (3) The nuclear polyhedra were not inactivated completely by the treatment of 110°C, 60 min. but the toxic crystals were not inactivated by 140-150°C, 60 min. On the other hand, the cytoplasmic polyhedra and the infectious flacherie viruses showed a low tolerance to the heat treatment.

**Matsumoto T, Matsubara F, Ohnishi M and Hayashiya K (1981). Researches on the application of aseptic rearing method to silkworm rearing. III. Microbial survey at the end of raising young larvae in the bioclean room. *J. Sericult. Sci. Jpn.* 50:359-365.**

The pathological studies were carried out when the sw larvae were reared on the artificial diet under an aseptic condition during the $1^{st}$ – $3^{rd}$ stages and then reared on mulberry shoot to the final stage. At the end of rearing in the bioclean room, the microbes such as bacteria, fungi and viruses were tested for the survey of health of the larvae.

(1) Microbes in the room air were checked by the Impinger method. Microbes in the larvae were examined by plate culturing method for bacteria and fungi, by microscopic observation of polyhedra for nuclear polyhedrosis virus (NPV) and cytoplasmic polyhedrosis virus (CPV), and by immunological diagnosis for flacherie virus (IFV). Two persons entered into the clean room

for the starting of rearing, bed cleaning, and supplying the food. (2) The number of viable microbes in the room counted in the range of 3-6/$m^3$ bacteria and 3.5 x 10-1.1 x $10^2$/$m^3$ fungal cells. *Bacillus, Staphylococcus, Pseudomonas, Proteus, Penicillium, Aspergillus, Cladosporium* and yeas were isolated from Impinger fluids. Microbes were counted mostly in summer. (3) Four microorganisms of *Staphylococcus, Enterococcus* and yeast were detected in larvae only in summer. No fungi and viruses of NPV, CPV and IFV were detected in every rearing season. (4) Thus certified larvae were followed by raising on mulberry shoot to the final stage. No diseased larvae were observed.

It was proposed by the authors that larvae reared on artificial food should be certified to be germ-free at the end of the raising in bio-clean room for stabilization of supplying of young larvae to the new Sericultural system.

**Furuta Y (1981). Pathogenicity and solubility of silkworm nuclear and cytoplasmic polyhedra treated with formaldehyde. *J. Sericult. Sci. Jpn.* 50:379-386. [Japanese]**

**Ishihara R and Nikaido H (1981). Buoyant density of *Nosema bombycis* (Nosematidae: Microsporida) spores. *J. Sericult. Sci. Jpn.* 50:457-458. [Japanese]**

**Watanabe H and Shimizu T (1981). A historical aspect on the epizootics of densonucleosis in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 50:472-477. [Japanese]**

## Vol. 51 1982

**Nagae T and Suzuki T (1982). The pathogenicity of *Streptococcus* bacteria isolated from the silkworm reared on an artificial diet. V. Effect of vitamins in the diet on the pathogenicity of *Streptococcus faecalis* to the silkworm. *J. Sericult. Sci. Jpn.* 51:40-45.**

Investigation was made on the effect of B vitamins added to the artificial diet on the pathogenicity of *Streptococcus faecalis* to silkworm larvae. *S. faecalis* M-8 was low pathogenic to larvae reared on the basal artifical diet without any B vitamin. The pathogenicity of *S. faecalis* M-8 increased markedly in the larvae reared on the diet including nicotinic acid, folic acid or biotin. The mineral optimal amount of B vitamins (ng/ml) required for the growth of *S. faecalis* was determined - Nicotinic acid, 25-250; folic acid, 0.5-2.5; biotin, 1.0-2.0, pantothenic acid, 50-150, pyridoxine, 1.0.

The variable pathogenicity of *S. faecalis* to the larva reared in an artificial diet was discussed in relation to mineral optimal amount of B vitamins required for the growth of the bacteria and the amount contained in the artificial diet.

**Kawakami K (1982). Estimation of fungus contamination by air sampling method in rearing room for the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:46-50.**

It is necessary for the safety production of cocoon in silkworm rearing to know an effect of fungicidal disinfection or a degree of microbial contamination in rearing room for the silkworm. Air sampling method by the Biotest RCS air centrifugal sampler was more effective than sedimentary sampling method with petridishes on collecting air born fungi contained in room air of rearing room for the silkworm. Fungi ranging from 10/100 liter to 7.5 x 10/100 liter were trapped by RCS air sampler from room air of silkworm rearing ouse. Both of fungi, *Penicillium* and *Aspergillus*, were found with high frequency in air sampling. A few colonies of *Cladosporium*, *Alternaria*, *Paerilomyces*, *Cephalosporium*, *Rhizopus* or *Mucor* were also detected in trappings. The biotest RCS centrifugal air sampler may be useful for estimating the microbial contamination in rooms or tools, such as in the silkworm rearing room with artificial diet, since it is highly efficient, battery powered, completely portable and easy to handle.

**Kawase S, Nakagai M, Bando H and Furuta Y (1982). Studies on the chemical properties of "flacherie virus isolted by Furuta" in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:58-65.**

Chemical properties on a small flacherie virus, "flacherie virus isolated by Furuta (FVF), were investigated and compared to those of Ina-flacherie virus (*Bombyx*-DNV). The purified FVF had a diameter of 22 nm and the buoyant density in CsCl was found to be 1.40. The nucleic acid in the intact virion was determined to be single stranded DNA by diphenylamine reaction, formal-dehyde reaction and acridine orange staining. Moreover, it was supposed that complementary single stranded DNA (plus and minus) was contained in separate virions, since the DNA was extracted as double stranded DNA under conditions of appropriate high salt buffer and elevated temperature. Four structural proteins, one major protein (VP1) with a molecular weight (MW) of about 50,000 and the other three minor proteins (VP2, VP3 and VP4) with MWs of about 57,000, 70,000 and 77,000 were found in FVF by SDS-PAGE. These compositions were closely similar to those of *Bombyx*-DNV. Immunological investigation using *Bombyx*-DNV antiserum also showed that both FVF and *Bombyx* DNV are identical virus, or both are closely similar strain to each other.

**Utsumi S and Nishimura T (1982). On the anti-*Streptococcus* protein (APS) in the digestive juice of silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:84-92.**

Growth of general bacteria, *eg, Bacillus thuringiensis, Pseudomonas aeruginosa, Staphylococcus aureus*, was inhibited under the alkaline condition of digestive juice of silkworm larvae, but that of the alkaline tolerant streptococci was not inhibited. Anti streptococcal activity was found in macromolecular parts obtained by means of the gel chromatography on sephadex G-75 from the fraction salting out of digestive juice. The active fraction was sensitive against the proteolytic enzymes and thermolabile at over 70°c for 15 min. The active fraction was sensitive against the proteolytic enzymes and thermolabile at over 70°C for 15 min. Therefore, the active substance seemed to be a macromolecular protein and was tentatively termed "Anti *Streptococcus* Protein (ASP). Anti bacterial spectrum of ASP exhibited the specific property namely growth of bacteria belonging to *Streptococcus* genus was inhibited exclusively, and mode of anti streptococcal action was considered to be bacteriostatic. The fifth instar larvae, administered orally the alkaline tolerant streptococci were reared continuously under unsuitable condition and fed on the malnutritional mulberry leaves. In such a case, many larvae tested were attacked with a disease of the intestinal flacherie, in which the multiplication of the administered bacteria with other bacteria was observed in their intestine. Accordingly, intestinal flacherie in the silkworm larvae seemed to have been occurred by multiplying the various bacteria in digestive organ, when the larvae had such a delicate condition as to lower the ASP activity of digestive fluid. In the digestive fluid, the alkaline tolerant streptococci were considered to be primary invaders, and to induce the acid production due to the bacterial multiplication. Secondary, the growth of invaders to be a kind of "Defensive Protein" occurring in the digestive fluid.

**Miyajima S (1982). Refractive index in haemolymph and gut juice of the silkworm infected with some viruses. *J. Sericult. Sci. Jpn.* 51:176-181.**

Refractive index in haemolymph and gut juice of the silkworm, *Bombyx mori*, infected with three viruses, *ie*, nuclear polyhedrosis virus (NPV), cytoplasmic polyhedrosis virus (CPV) or flacherie virus (FV) is described. The results obtained are as follows.

1. **Refractive index in haemolymph:** In the case of oral inoculation with CPV to the 5$^{th}$ instar larvae, refractive index in the haemolymph showed lowered haemolymph values as compared to the healthy larvae, and from 4 days later the difference increased. When silkworm larvae were inoculated with NPV *per os*, the index of infected larvae showed a little low value

comparing with control, and the both values increased in parallel. When FV was inoculated *per os* into 4th instar larvae, the difference of the values between inoculated and control became large on 8-9 days after FV inoculation. When the 5th instar larvae treated at 5°C for 48 h and showed the same symptoms of cytoplasmic polyhedrosis were compared with control, the index was found to be lower than control.

2. **Refractive index in gut juice:** In the case of peroral inoculation with CPV, the difference of the index of gut juice was not found until 3 days after CPV inoculation, but 4-6 days after the inoculation, the difference became a little large. When the larvae were inoculated with NPV or FV, or treated with cold treatment, the values in these virus inoculated larvae did not show any difference as compared to those of control.

**Iizuka T, Abe K, Faust RM, Ohba M and Bulla LA (1982). Isolation of Covalently Circular DNA from *Bacillus thuringiensis* subspp. *Tohokuensis, kumamotoensis* and *tochigiensis*. *J. Sericult. Sci. Jpn.* 51:209-217 [English]**

Three new serotypes of *B. thuringiensis* were examined for the presence of covalently closed circular (CCC) DNA molecules using a procedure which optimizes lysis or the normally lysozyme resistant vegetative cells. The results obtained can be summarized as follows:

Two of the three subspecies of *B. thuringiensis* contained CCC DNA molecules of several sizes that were readily visualized with agarose gel electrophoresis and that ranged from 120 x $10^6$ to approximately 2 x $10^2$ daltons, depending on the strain. A determination of the numbers of plasmid DNAs based on agarose gel profiles revealed there were 3 for subsp. *tohokuensis*, 4 for subsp. *kumamotoensis* and non for subsp. *tochigiensis*. *B. thuringiensis* subsp. *kumamotoensis* harboured a giant CCC DNA above molecular weight of 120 x $10^6$ daltons, however both subsp. *tohokuensis* and *kumamotoensis* each harboured 3 relatively small CCC DNAs of similar sizes. The absence of extrachromosomal DNA elements in subsp. *tochigiensis* in this experiment indicates the countrary with results of many investigations that the production of parasporal body is accompanied by the presence of plasmic DNA. Transformation studies to determine the genetic fuctions of plasmid DNA are in progress.

**Seki T, Abe K, Tsutsui R and Watanabe T (1982). Isolation and partial purification of an enzyme contained in the silkworm digestive juice solubilizing *Bacillus thuringiensis* toxic crystal. *J. Sericult. Sci. Jpn.* 51:279-285.**

The toxic crystal obtained from *Bacillus thuringiensis* was solubilized by the digestive juice of the silkworm larvae. The solubilizing activity of the enzyme in the silkworm digestive juice was assayed by estimating the absorbance at 280 nm of toxic solution. An enzyme solubilizing toxic crystal in the digestive juice of silkworm larvae was isolated and purified 70 fold by methyl alcohol treatment, gel filtration on Sephadex G-75 and column chromatography on DEAE-Sephadex A-50. The enzyme also showed the proteolytic activity against casein, and the solubilization activity was similar to the proteolytic activity in optimum pH, PH stability and thermal stability. These results suggested that the enzyme solubilizing toxic crystal in the silkworm digestive juice had also a protease like activity.

**Seki T, Faust RM, Abe K, Bulla LA, Tsutsui R and Watanabe T (1982). Biochemical Action of the *Bacillus thuringiensis* subsp. *dendrolimus* δ-Endotoxin to the Silkworm Larva, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:289-304. [English]**

The molecular mode of action of the δ-Endotoxin produced by *B. thuringiensis* subsp. *dendrolimus* at the subcellular level was investigated. Parasporal crystals contained a toxic substance unit that had an apparent molecular weight of approximately 66,000 when solubilized with protease or midgut juice from larvae of *B. mori* (L.). In addition to this subunit, a large

polypeptide that had an apparent molecular weight of about 2,30,000 and another toxic subunit with and apparent molecular weight of 33,000 were isolated from parasporal crystals treated with midgut juice of NaOH. The toxic subunit of molecular weight 66,000 caused inhibition of succinate dehydrogenase and cytochrome c oxidase activities in midgut mitochondrial preparations of *B. mori* larvae. The toxin had no significant effect on these activities in midgut mictochondrial preparations of *P. Americana* (L.). The toxic subunit also caused a reduction of midgut ATP levels *in vivo*. *In vitro* effects on cytochrome c by all the fractionated toxin moieties from the crystalline inclusion bodies suggest that the toxin has proton carrying properties not unlike uncoupling agents against promoting electrogenic $H^+$ transport across mitochondrial membranes with release of respiration, effects on cytochrome c resulted in spectral shift and reduction of its oxidized form. The experimental results indicate that the *B. thuringiensis* δ-endotoxin can interfere with subcellular respiratory enzymes and carriers and may lead to uncoupling of oxidative phosphorylation (or interfere with ATP formation) and dissociation of cellular oxidation from energy production as postulated by other workers.

**Faust RM, Adams JR, Abe K, Iizuka T and Bulla LA (1982). Comparative Morphology and Size Distribution of the Parasporal Crystals from Various Strains of *Bacillus thuringiensis*. *J. Sericult. Sci. Jpn.* 51:316-324. [English]**

Thirty three strains of *B. thuringiensis* representing 24 different H-antigen serotypes and 2 non H-antigenic strains were examined by electron microscopy and their parasporal inclusions were compared for differences in morphology and size. In general, the parasporal crystals in the various strains of *B. thuringiensis* were found to be bipyramidal in shape, however, some strains produced crystals that were cuboidal, rhomboidal or irregular in shape. A wide variation in size of parasporal crystals exists among the various strains of *B. thuringiensis* with a continuum of sizes that range in length from relatively small (0.6 μm) to unusually large 1.8 μm crystals. Widths varied from 0.3-0.9 μm. In at least 10 strains of *B. thuringiensis,* inclusion bodies other than the parasporal crystals were produced that often could be observed embedded in the parasporal crystal and when freed, these bodies had shapes that were usually cuboidal, although spheroidal and ovoidal shapes also were observed. Freeing of the inclusion bodies from the parasporal crystals left cavities at the site of the embedded body in the crystal.

**Mikuni T, Kawakami K and Nakayama M (1982). Survival of an entomogenous fungus, *Metarhizium anisopliae,* causing the muscardine disease of the silkworm, *Bombyx mori,* in the soil of mulberry plantation. *J. Sericult. Sci. Jpn.* 51:325-331.**

To know the primary infectious sources for the infection to the insects, we investigated the survival, germination and multiplication of *Metarhizium anisopliae* in the soil of mulberry plantation. The fungus, *M. anisopliae,* was detected in number of $10^2$-$10^3$/g soil throughout the year by the dilution plate method using CRCC agar from the soil of ordinary mulberry plantation. However, seasonal changes in number of the fungus were not found in the field test. The fungus isolates detected from the soil of the field were pathogenic to silkworm larvae almost as of high pathogenicity as the isolate from the diseased silkworm. In the soil infested respectively with conidia of three isolates, each isolate of *M. Anisopliae* was detected in number of $10^2$-$10^4$/g soil for 25 months in the field test. Seasonal fluctuation of density of the fungus was not recognized in the soil examined. The conidia of *M. anisopliae* germinated easily in untreated soil from the fields after 20 h at 25°C and new conidia were produced in chain at the tip of single for small phialide after 48 h at 25°C. The conidia taken from conidiophores of *M. anisopliae* survived only 10-30 days at 25°C in laboratory test. Longevity of this fungus conidia was shorter than other entomogenous fungi, *Beauveria bassiana, B. tenella* and *Aspergillus flavus*. The results obtained clearly suggest that the fungus *M. anisopliae* is distributed widely in the soil of mulberry plantation and the fungus can grow in the soil.

**Kawase S and Miyajima S (1982). Inhibitory effect of guanidine on the incidence of infectious flacherie of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:341-345.**

Inhibitory effect of guanidine hydrochloride (GH) and 2-(α0hydroxybenzyl) benzimidazole (HBB), specific inhibitors of some picorna viruses was investigated on the incidence of infectious flacherie of the silkworm, which is due to a picorna-like virus, infectious flacherie virus (IFV). The progression of the infectious flacherie caused by IFV inoculation was inhibited markedly when the larvae were fed with aqueous solution of 0.01% GH or several other guanidine salts immediately after or before the IFV inoculation, and there after fed with guanidine treated leaves every days. However, no inhibitory effect of guanidine was observed when guanidine treated leaves were fed two days, or after more than 42 h of IFV inoculation. GH did not inhibit IFV *in vitro*. In the case of HBB, no significant inhibitory effect was shown on the incidence of infectious flacherie.

**Shimizu S (1982). Enzyme-linked immunosorbent assay for the detection of the flacherie virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:370-373.**

An enzyme linked immunosorbent assay (ELISA) was developed for the detection of the flacherie virus of the silkworm, *Bombyx mori*. This technique proved to be specific for the flacherie virus and virus protein of 3 ng/ml could be detected. The sensitivity of ELISA was 6,000, 1,700 and 200 times higher than that of gel immunodiffusion, ring test, and latex agglutination, respectively. Addition of larval extracts did not inhibit the virus-antibody reaction in this assay system. The amount of the virus in the infected larvae estimated by ELISA was proportional to the infectivity titer of the virus.

**Nakagaki M and Kawase S (1982). Capsid Structure of the Densonucleosis Virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:420-424. [English]**

The capsid structure of densonucleosis virus of the silkworm, *Bombyx mori* was investigated by electron microscopy using negative staining technique. In the high-magnification micrographs from highly purified virion and using rotation technique, it was recognized that the virion had two fold, three fold and five fold axes of symmetry, and there was good agreement between the virus particle and the twelve capsomer model. The globular structure which were compatible with the theoretical size of such capsomers existed in the virus preparation. We suggest, therefore, that the surface of *Bombyx* DNV particle has a form of an icosahedron formed from twelve capsomers.

**Nitta M, Maeda S and Kawai T (1982). Characterisation of a protease in the nuclear polyhedra of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 51:430-434.**

Several reports have detected the alkaline protease in the inclusion bodies of baculoviruses (nuclear polyhedrosis virus and granulosis virus). The resent study confirmed the presence of alkaline protease in the nuclear polyhedra of the silkworm, *Bombyx mori*, and characteristics of the protease. When the nuclear polyhedra were exposed to alkaline solution, a proteolytic enzyme in the polyhedra degraded the major structural proteins of polyhedra into proteins with the lower molecular weight. The proteolytic activity showed a temperature optimum of 45°c to 50°C and a pH optimum of 10.5 to 11.2. The activity was inhibited by L-tosylamide phenylalanine chloromethyl ketone (TPCK), L-tosyl lysine chloromethyl ketone (TLCK) and metallic ions such as $Hg^{2+}$ and $Cu^{2+}$. These results suggested that the proteolytic enzyme in the nuclear polyhedra was similar in characteristics to a protease in the digestive juice of the silkworm.

**Kusunoki J and Watanabe H (1982). Changes in the haemolymph pH and specific gravity of the silkworm larvae, *Bombyx mori*, infected with *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 51:447-448. [Japanese]**

**Kusunoki J and Watanabe H (1982). Changes in the free amino acid composition of the haemolymph from the fifth instar larvae, *Bombyx mori*, infected with *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 51:517-522.**

Changes in the free amino acid composition of haemolymph of silkworm larvae infected with *Beauveria bassiana* were examined. At a later stage of the infection with *B. bassiana*, contents of glutamic acid, cysteine and tyrosine in the haemolymph of diseased larvae were markedly increased as compared to those of the healthy larvae, while contents of aspartic acid, glutamine, praline, alanine, valine, isoleucine and arginine were decreased. Free amino acid analysis of the liquid medium on which *B. bassiana* was cultured for three days indicated that *B. bassiana* required much amounts of glutamic acid, lanthionine, valine, leucine, lysine and arginine during the growth and released some amounts of serine, ornithine, and methionine into the medium. These results suggested that the changes in the free amino acid composition of the diseased haemolymph were not directly affected by amino acid metabolism of *B. bassiana* multiplied in the haemolymph but mainly took place in a consequence of pathophysiological changes of the host larva.

**Yanagita T (1982). On amino sugars isolated from silkworm larvae by infection of *Aspergillus flavus-oryzae* fungi. *J. Sericult. Sci. Jpn.* 51:528-532.**

The newly hatched larvae were inoculated with *Aspergillus flavus-oryzae* and amino sugars were extracted from the larval body. Those amino sugars were fractionized four peaks by column chromatography with Dowex50-X8; one peak by deionized water and the other three peaks by 0.3N HCl. The fractionation by Dowex50-X8 column showed the similar pattern in spite of pathogenicity of *Aspergillus* fungi inoculated to larvae.

**Yanagita T and Iwashita Y (1982). Discharge and regeneration of the midgut epithelial cells in the silkworm larvae infected with *Aspergillus flavus-oryzae* fungi. *J. Sericult. Sci. Jpn.* 51:533-536.**

Histopathological observation on the midgut epithelium of the silkworm larvae infected by *Aspergillus flavus-oryzae* fungi was made. When the hyphae penetrated into the body cavity was further arrived at the midgut, the cylindrical cells were partially discharged from the basement membrane into the gut lumen and they were replaced by newly generated epithelial cells. Discharge and regeneration of the cells occurred in the midgut were followed by hyphal invasion into the gut tissue.

## Vol. 52 1983

**Yanagita T and Iwashita Y (1983). Scanning electron microscopic observation on the infection of the integument of the silkworm larvae by *Aspergillus flavus-oryzae* fungi. *J. Sericult. Sci. Jpn.* 52:41-46.**

The infection of the integument of the silkworm larvae by *Aspergillus flavus-oryzae* fungi was histopathologically observed by scanning electron microscopy. Germ tube or hyphae of *Aspergillus flavus-oryzae* fungi when invade the integument formed a suctorial or humpy appressoria, on the host integument. Although appressoria were fomed both in the low and the high pathogenic strain, the low pathogenic hyphae were significantly delayed or unable to penetrate into the larval integument. Penetration into the cuticle was observed on their regions of

body surface, such as the indent part of wrinkle, the top of leg, the connecting part of seta and around the parts of the spiracle.

**Ono K and Watanabe H (1983). Distribution and serological identification of *Bacillus thuringiensis* in Japan. *J. Sericult. Sci. Jpn.* 52:47-50.**

Distribution of *Bacillus thuringiensis* in the dust from Sericultural farms was investigated and the serotypes of the isolates were examined. *B. thuringiensis* was isolated from 14 out of 233 farms in various districts in Japan. All of these isolates were identified to belong to the serotype 3a (subsp. *alesti)*, showing high toxicity to the silkworm larvae.

**Kuroda S and Watanabe H (1983). Changes in the concentration of α-ketoglutaric acid and free amino acids in the haemolymph of the silkworm *Bombyx mori*, infected with a nuclear-polyhedrosis virus. *J. Sericult. Sci. Jpn.* 52:172-176.**

Changes in the concentration of α-ketoglutaric acid (α-KG) and free amino acids in the haemolymph were investigated on the female silkworm larvae, *Bombyx mori*, during the course of nuclear polyhedrosis in the 5$^{th}$ instar. A marked increase in concentration was recognized on the 4$^{th}$ to 5$^{th}$ days after virus inoculation. On the other hand, the concentration of most free amino acids in the haemolymph such as methionine, isoleucine, arginine, praline and valine, etc., increased significantly on the 4$^{th}$ day post inoculation and on the following day, the majority of amino acids turned decrease in the concentration. Whereas some other amino acid concentrations continued to increase. Thus a remarkable change in both α-KG and amino acid concentrations in the larval haemolymph took place at a late stage of virus infection when the diseased haemolymph was turned white in colour by polyhedron formation.

**Furuta Y (1983). Multiplication of the infectious flacherie virus and the densonucleosis virus in cultured silkworm embryos. *J. Sericult. Sci. Jpn.* 52:245-246. [Japanese]**

**Kobayashi M, Tanaka K and Ishikawa M (1983). Relationship between the occurrence of the nuclear polyhedrosis in *Antheraea yamamai* and contamination of the polyhedra in the field soil. *J. Sericult. Sci. Jpn.* 52:324-329.**

The occurrence of the nuclear polyhedrosis and soil pollution by the pathogen was investigated by a series of experiments to explicate the factors that cause the bad cocoon crop of *Antherae yamamai*. Polyhedra isolated from the soil of rearing field showed pathogenicity. Scanning electron microscopic examination revealed a close morphological similarity between the polyhedra isolated from the infected larvae of *Antheraea yamamai* and the polyhedra isolated from the soil. Close similarity was also noted by measuring the optical density at 260 nm between the virus particles isolated from the infected larvae of *Antheraea yamamai* and those obtained from the soil. Above results suggested that the polyhedra contained in feces or dead bodies of infected larvae were accumulated in the soil surface layer as a source of infection. Prevalence of the nuclear polyhedrosis in *Antheraea yamamai* was considered to be mainly caused by contamination of the field and induced by meteorological conditions.

**Miyajima S and Kawase S (1983). Inhibitory dose of guanidine and its application interval on the incidence of infectious flacherie of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 52:357-358. [Japanese]**

**Miyajima S, Washida S and Kawase S (1983). Inhibitory effect of guanidine on the incidence of infectious flacherie of the silkworm, *Bombyx mori*, reared on artificial diet. *J. Sericult. Sci. Jpn.* 52:390-393.**

It was reported in an earlier paper that the progression of theflacherie caused by infectious flacherie virus (IFV) inoculation was inhibited markedly when the silkworm larvae were fed with aqueous solution of 0.01% guanidine hydrochloride (GH), but the inhibitory effect of GH disappeared when IFV inoculated larvae were reared on an artificial diet. In order to clarify the reason, several different artificial diets which were removed one component from the basal composition were used. When an artificial diet including GH and without gallic acid was applied to the IFV inoculated larvae, the incidence of flacherie was inhibited markedly. From these results, it was suggested that gallic acid was a main factor participated in the disappearance of the inhibitory effect of GH on the incidence of flacherie of the silkworm.

**Seki H and Iwashita Y (1983). Histopathological featues and pathogenicity of a densonucleosis virus of the silkworm, *Bombyx mori*, isolated from Sericultural farms in Yamanashi Prefecture. *J. Sericult. Sci. Jpn.* 52:400-405.**

A virus isolated from diseased silkworm larvae collected at Sericultural farms in Yamanashi prefecture caused a high larval mortality in two hybrids of silkworm strains employed. Electron and light microscopic observations of the midgut epithelium of the diseased larvae showed the aggregation of a great number of spherical virions, 20 nm in diameter, in the infected nuclei of columnar cells, replacing completely the whole nuclear materials. In addition, the infected nuclei became hypertrophied and showed strongly Feulgen positive reaction and methyl green staining. The present observations suggest that the newly isolated virus belongs to the group of densonucleosis viruses, and this was designated as “Yamanashi isolate”.

**Matsubara F, Go Y, Mori H and Ogashira H (1983). Difference in the susceptibility of germfree silkworms at larval stages to the oral infection with nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 52:419-424.**

When the silkworms were administered orally with nuclear polyhedra, the susceptibility to nuclear polyhedrosis virus (NPV) varied at different stages, and the susceptibility increased with larval development. However, when administered orally with grasery juice (haemolymph of jaundice larva), the susceptibility of the silkworms to NPV decreased with growth from the 1$^{st}$ to the 3$^{rd}$ instars, but increased from 4$^{th}$ to the 5$^{th}$ instars. No difference in the susceptibility to NPV was recognized between larvae reared under aseptic and non-aseptic conditions.

**Shimizu S (1983). Morphology of the formation of blastospores in *Paecilomyces fumosoroseus*. *J. Sericult. Sci. Jpn.* 52:443-450.**

Morphology on the formation of blastospores of *P. fumosoroseus* was investigated and following results were obtained. L-broth was found to be a suitable medium for the production of blastospores of *P. fumosoroseus* and the maintenance of their form. The medium composition of of L-broth in sucrose 2%, peptone 1%, NaCl 0.5%, yeast extract 0.3% (pH 6.8-7.0). In L-broth, blastospores were formed as buds on elongated hyphae subsequently on short hyphae at the logarithmic phase of the fungal growth. Blastospores were oval in shape, 2.5-7.5 μ in length and 2-3.5 μ in width. *P. fumosoroseus* multiplied in the haemolymph of the silkworm, *Bombyx mori*, by budding of blastospores.

**Utsumi S (1983). Effect of food condition on the anti-*Streptococcus* activity in the digestive juice of the silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 52:537-544.**

To clarify the anti-bacterial action as a defense mechanism in the digestive juice of the silkworm, the properties of anti-*Streptococcus* protein (ASP) and the effect of dietary conditions on the anti-bacterial activity of ASP were investigated. ASP was found in the precipitates by ammonium sulfate at the concentrations of 0-30% and 30-70%, but not 70-100%. A single peak of ASP in both precipitates was obtained by Sephadex G-75 column chromatography, whereas

two active peaks were obtained by DEAE-cellulose chromatography. ASP purified by Sephadex G-75 chromatography was highest in larvae reared on mulberry leaves in spring, in the 175 time dilution the sample showed the activity to inhibit bacterial growth completely. In larvae reared on the leaves in later autumn, the 59 time dilution was effective. However, in larvae, which were reared on artificial diet by the end of the $4^{th}$ instar and no leaves thereafter, only 18 time dilution was effective. When reared on the leaves stored for a long period, no dilution was effective. Thus, the presence of two forms is at least suggested and their production is seemed to be controlled by food condition.

**Kawase S, Bando H and You-Min Cai (1983). Purification of a densonucleosis virus from silkworm feces. *J. Sericult. Sci. Jpn.* 52:547-548. [Japanese]**

## Vol. 53 1984

**Utsumi S (1984). Chemical Characteristics of a Previously Undescribed Densonucleosis Virus Isolated from the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:33-40. [English]**

A small spherical virus of 23-24 nm in diameter which multiplied in the nuclei of columnar cells of the midgut epithelium was isolated from diseased larvae of the silkworm, *Bombyx mori*, and the properties of the viral proteins and nucleic acid were characterised. By sodium dedecyl sulphate-polyacrylamide gel electrophoresis, four structural proteins (P1, P2, P3 and P4) were detected and their molecular weights were estimated from relative mobilities as 121,000, 116,000, 53,000 and 50,000 daltons, respectively. Peptide mapping revealed that the P1 was similar to P2, P3 and P4. The virus contained single stranded (ss) DNA. When the DNA was extracted in a low salt buffer, it showed properties of a single stranded molecule, while a double stranded (ds) molecule was extracted under a high salt condition. The ssDNA molecule had a molecular weight of about $1.99 \times 10^6$ by agarose gel electrophoresis. These data suggested that the newly isolated virus belonged to the *Densovirus* group of Parvoviridae. The newly isolated virus differed in molecular weights of structural proteins and DNA from the previously described densovirus from *Bombyx mori* and the two viruses were not serologically related.

**Seki T, Abe K, Tsutsui R and Watanabe T (1984). Purification and properties of toxic protein from crystal of *Basillus thuringiensis*. *J. Sericult. Sci. Jpn.* 53:41-47.**

The crystal of *Bacillus thuringiensis* subsp. *dendrolimus* strain T84A1 was dissolved by the purified protease in the silkworm digestive juice. The soluble toxin was unstable in 0.2 M Tris-HCl buffer or 1/15 M phosphate buffer, but stabilized by adding 1mM EDTA, 0.1 M glycine and 0.1-2 M urea in 0.2 M Tris-HCl buffer (ph 8.5). Also, the toxic protein was stable in 0.2 M glycine-NaOH buffer (pH 8.5-9.0). The toxic protein was purified by means of Sephadex G-75 gel filtration and DEAE cellulose chromatography. The $LD_{50}$ of the soluble toxin was 0.29 μg/g. The $LD_{50}$ of the purified toxin by gel filtration or by DEAE cellulose chromatography was 0.14 μg/g. The toxic protein was purified 2.2 fold. The toxic protein gave a single band in slab gel electrophoresis of SDS-polyacrylamide. Molecular weight of the toxic protein was estimated to be 66,000 by SDS-PAGE.

**Mike A, Ohwaki M, Fukada T and Miyajima S (1984). Preparation of monoclonal antibodies to the *Bombyx mori* cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn.* 53:59-63.**

Monoclonal antibodies were produced to the *Bombyx mori* cytoplasmic polyhedrosis virus which forms polyhedra of tetragonal shape (CPV-T). Three hybridoma antibodies, termed S11 (IgG1), S21 (IgG1), S91 (IgG2a) were reactive with two strains of CPV, CPV-T and CPV-H which forms polyhedra of hexagonal shape, but unreactive with *Bombyx mori* nuclear

polyhedrosis virus (NPV) by indirect enzyme linked immunosorbent assay (ELISA). Both S11 and S21 could neutralize CPV-T but not NPV.

**Seki H (1984). The serological properties and infectivity of Yamanashi isolate of the silkworm densonucleosis virus. *J. Sericult. Sci. Jpn.* 53:69-71.**

The Yamanashi isolate of the silkworm densonucleosis virus did not react with the antisera against the Ina isolate and the small flacherie virus in immunodiffusion tests. These viruses did not appear to share any antigen with the Yamanashi isolate. The virus did not infect larvae of N124 but did infect larvae of seven other varieties. Accordingly, the Yamanashi isolate differed from the Ina isolate in the infectivity to silkworm varieties.

**Inoue H and Mitsuhashi J (1984). A *Bombyx mori* Cell Line Susceptible to a Nuclear Polyhedrosis Virus. *J. Sericult. Sci. Jpn.* 53:108-113. [English]**

Embryonic cells from the “Kuroko” strain, a genetically well defined strain of the silkworm, *Bombyx mori* were cultured *in vitro*. In primary cultures, epithelial like cells migrated and multiplied markedly. They contained large granules around their nuclei. This type of cells could be subcultured continuously, though their growth was comparatively slow. This cell line was found to be susceptible to *B. mori* nuclear polyhedrosis virus.

**Noguchi Y (1984). Histopathological observations on the midgut epithelia of lepidopterous insects naturally recovered from infection by a cytoplasmic-polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:141-145.**

Electron microscopical observations were performed on the midgut epithelia from *Hyphantria cunea* and *Orgyia thyellina* larvae infected with a *Bombyx* cytoplasmic polyhedrosis virus (CPV). In the midgut epithelial cells of those insects, empty capsids of BmCPV were increased in number, whereas normal virus replication and development of virogenic stroma were suppressed. In the renewed epithelial cells which had been regenerated after the infected cells were discharged into the gut lumen, no histopathological lesion by virus infection was observed. In a few cells immediately after regeneration, however, virogenic stroma of small size were observed where empty capsids were assembled. Further, polyhedron protein and BmPCV were not detected in the renewed cells by immunoflourescence technique. These results suggested that the renewed cells might acquire an ability to prevent the replication of BmCPV.

**Nitta M and Watanabe H (1984). Effects of formalin on characteristics of nuclear polyhedra of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:146-150.**

A formalin treatment made nuclear polyhedra of the silkworm, *Bombyx mori*, hard to soluble in alkaline solution or digestive juice from the larvae. SDS-PAGE of the formalin treated polyhedral protein revealed a pattern indicating fixation of protein. Alkaline protease present in the polyhedra was also inactivated by the formalin treatment. However, the solubility and the protease activity of polyhedra were recovered when they were kept in distilled water or 0.05 M Tris-HCl buffer (pH 7.6)-0.05 M NaCl.

**Watanabe H (1984). Effects of an antibiotic on polyhedrosis-virus infection in the silkworm, *Bombyx mori* reared on an artificial diet. *J. Sericult. Sci. Jpn.* 53:160-164.**

The susceptibility and the lethal time of infection by a nuclear polyhedrosis virus (NPV) were almost the same between silkworm larvae reared on artificial diets with or without containing of an antibiotic, chloramphenicol, indicating the antibiotic in artificial diet had no effect on NPV infection and multiplication in the fed larvae. On the other hand, the antibiotic in diet was great effective to prolong the lethal time of the fed larvae infected with a cytoplasmic

polyhedrosis virus (CPV). The prolongation of life was mainly due to the fact that the antibiotic in diet almost suppressed the abnormal multiplication of intestinal bacteria in the midgut lumen, which as usually accompanied by CPV infection.

**Matsubara F, Yu-Liang Wu, Mori H and Oogashira H (1984). Difference in susceptibility of germ free silkworm at each instar to NPV after cold treatment. *J. Sericult. Sci. Jpn.* 53:205-209.**

Changes in the susceptibility of each instar larva of the silkworm, *Bombyx mori* to NPV after cold treatment (5°C, 24 h) were studied under an aseptic condition. Inoculum virus was nuclear polyhedrosis virus filtered through a 0.45 μm Millipore filter after the dissolution of nuclear polyhedra in alkali. Although newly hatched larvae were not sensitive to cold treatment, susceptibility to NPV after cold treatment increased with larval age from the 2nd to 5th instars. Infective NPV was detected in the haemolymph of the 5th instar larvae at 6 h after the peroral administration with nuclear polyhedra following cold treatment.

**Kawakami K and Mikuni T (1984). On the susceptibility of larvae of the silkworm, *Bombyx mori*, reared on artificial diets to the infection with muscardine fungi. *J. Sericult. Sci. Jpn.* 53:245-249.**

In view of control of muscardine diseases of the silkworm, the susceptibility of larvae, reared either on artificial diets or mulberry leaves, to fungus infection was investigated. Two kinds of artificial diets were used for silkworm rearing in the experiment. Fungus species used were *Beauveria bassiana*, *Metarhizium anisopliae* and *Nomuraea rileyi*. Susceptibilities of larvae to fungus infected were tested by means of topical application of fungus conidia on integument of 3rd instar or 4th instar larvae, injection of conidial suspension into body cavity of 4th instar larvae, and slide culture for germination of fungus conidia in the blood from the larvae. The results of thee experiments showed that there was no difference in the susceptibility to infection with muscardine fungi between the larvae reared on artificial diets and those reared on mulberry leaves. It is thus necessary to carry out the control of muscardine diseases by usual methods.

**Noguchi Y and Yamaguchi K (1984). An acquired resistance to virus infection in the lepidopterous insects naturally cured from *Bombyx* cytoplasmic polyhedrosis. *J. Sericult. Sci. Jpn.* 53:325-330.**

After naturally recovered from the disease caused by BmCPV, *Hyphantria cunea* and *Orgyia thyellina* acquired a high resistance to the same virus species, BmCPV, but their susceptibilities to other species of CPVs, NPVs and a granulosis virus were not different from those of the larvae uninfected previously with BmCPV. Electron microscopical observation revealed that no sign of the replication of the secondary inoculated BmCPV was detected in the midgut epithelium of the larvae which have been cured from BmCPV, but secondary inoculated CPVs of the other species multiplied normally. These results suggested that the high resistance to virus infection acquired in the insects after natural recovery from Bm cytoplasmic polyhedrosis was specific against the infection of the same virus species.

**Kawase S, You-Min Cai, Bando H and Seki H (1984). Chemical properties of the Yamanashi isolate of the *Bombyx* densonucleosis virus. *J. Sericult. Sci. Jpn.* 53:341-347.**

Chemical properties of a small spherical virus, which was recently isolated in Yamanashi prefecture, were investigated and compared with those of Ina isolate of BmDNV. Negative stained virions of Yamanashi isolate had a diameter of 24 nm. A few of them had several projection like structure. The nucleic acid in the intact virion was determined to be single stranded 9ss) DNA by diphenylamine reaction, formaldehyde reaction and acridine orange staining. Since the DNA was extracted as double stranded (ds) DNA under conditions of

appropriate high salt or even fairly low salt buffer, it was presumed that complementary ssDNA was contained in separate virions, and two complementary ssDNAs in different particles were joined together by complementary base pairing. The viral DNA of Yamanashi isolate was resolved in agarose gel electrophoresis into two closely migrating bands (MW: 4.0 x $10^6$ (DNA I) and 3.8 x $10^6$ (DNA II) respectively as dsDNA), while only one band was formed for the viral DNA of Ina isolate. When both DNA I and II were digested with several restriction endonucleases, the electrophorograms of these fragments were clearly different from each other. Using SDS-PAGE, six structural proteins were detected from dissociated highly purified virions. Yamanashi isolate may consist of two different DNVs, which have three structural proteins and are clearly different from Ina isolate of BmDNV.

**Kadoya T, Yamashita O and Kawase S (1984). Carbohydrate changes in haemolymph and midgut epithelium of the silkworm, *Bombyx mori*, during the course of cytoplasmic polyhedrosis. *J. Sericult. Sci. Jpn.* 53:352-357.**

The daily changes in the concentrations of sugar and glycogen in the haemolymph and midgut epithelium were investigated in the 5$^{th}$ instar silkworm larvae infected with cytoplasmic polyhedrosis virus (CPV). In the diseased larvae, a distinct decrease of haemolymph sugar concentration was observed comparing to control larvae during the course of cytoplasmic polyhedrosis. However, the pattern was similar to that of starved larvae. Changes in sugar contents in CPV infected midguts were almost same as those of control or starved larvae. On the other hand, significant increase in glycogen contents in midgut epithelium infected with CPV was observed comparing to those of control or starved larvae during the course of cytoplasmic polyhedrosis. The results suggest that glycogen metabolism in midgut epithelium is affected more directly by the CPV multiplication.

**Fujiwara T and Kagawa T (1984). Control of *Nosema bombycis* prasitizing silkworm eggs by treatment with hydrochloric acid or exposure to various temperatures. *J. Sericult. Sci. Jpn.* 53:394-397.**

*Nosema bombycis* parasitizing the diapause eggs of the silkworm was effectively controlled when the eggs obtained 48 h after oviposition were stored at 5°C for 30 days and then treated with 20% HCl at 48°C for 5 min. The parasites in the non diapause eggs were also sensitive to similar treatment when the eggs obtained 48 h after oviposition were stored at 5°C for 30 days. The acid treatment could be replaced by the hot water treatment at 46°C for 4 min. No harmful effect of the treatment on the normal development of the silkworm embryos was observed.

**Fujiwara T (1984). A *Pleistophora* like microsporidian isolate from the sislkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:398-402.**

A few female moths of the silkworm were found infected with a *Pleistophora* like Microsporidian in the spring of 1970 in Chiba prefecture. Generally, incidence of the disease was extremely low in the spring rearing season and slightly increased in the fall. The parasite was new to the silkworm, and invaded into muscels, fat bodies, Malpighian tubes and silk glands of the larvae. Pansporoblasts were observed in the infected cells, and later, sixteen or more spores were formed in a cyst. Spores were oval in shape (5.06 x 2.97 μm) and polar filament was about 140 μm in length.

**Shimizu T and Watanabe H (1984). Failure to harvest cocoons attributed to an epizootic of densonucleosis caued by a new strain of the virus in sericultural farms. *J. Sericult. Sci. Jpn.* 53:436-440.**

In the late autumn rearing season of 1983, young silkworm larvae which had been reared until the second instar stage in a cooperative farm in the northern part of Nagano prefecture were

distributed for further rearing to 119 sericultural farms around the area. In the late larval stages, however, a large number of larvae died with flaccidity symptoms in every farm and no cocoons were harvested. The diseased larvae collected from farms were subjected to serological diagnosis in applying the fluorescent antibody technique. It was shown that most of the larvae were infected with the Saku strain of the densonucleosis virus, which had been recently isolated and characterised and found to be different from the Ina strain of the densonucleosis virus which had been previously described. The virus infection which may have affected young larvae in the cooperative farm had presumably spread horizontally to Sericultural farms around the area by way of silkworm distribution. This is the first record of an epizootic of densonucleosis caused by the Saku strain of the virus.

**Fujiwara T (1984). *Thelohania* sp. (Microsporidia: Thelohaniidae) isolated from the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:459-460. [Japanese]**

**Seki H (1984). Mode of inheritance of the resistance to the infection with the densonucleosis virus (Yamanashi isolate) in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:472-475.**

In order to analyse the mode of inheritance of the resistance to the infection with the virus (Yamanashi isolate), some experiments were carried out by using N124 as a resistant race and C124 as a susceptible race.

F1 and F2 hybrids were slightly more susceptible that C124. The back crossed progeny of C124 to F1 hybrids showed a susceptibility similar to that of C124. On the other hand, the backcrossed progeny of N124 to the F1 was highly resistant. In the relationship between log dosage-probit infectivity, the curve drawn on the basis of the experimental data coincided with the theoretical curve, on the assumption that one major gene controls the resistance. As mentioned above, it is suggested that the resistance to the Yamanashi isolate is controlled by one recessive major gene.

**Matsubara F, You-Liang Wu, Mori H and Oogashira H (1984). Changes in the resistance to the infection with NPV induced by low and high temperature treatment in the germ-free silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 53:538-542.**

Changes in the resistance to the infection with NPV was investigated by subjecting 4$^{th}$ and 5$^{th}$ instar larvae to low temperature (5 and 10°C) and high temperature (33, 35 and 37°C) treatments for various periods of time immediately after ecdysis. Although the resistance of the 5$^{th}$ instar larvae to NPV was not reduced by treatments at 10 and 33°C for 24 h, the larvae became markedly susceptible to NPV after treatments at 5, 7, and 37°C for 24 h. The resistance of the 4$^{th}$ instar larvae to NPV was reduced, when the low temperature (5°C) was applied for 3 h, whereas the resistance of the 5$^{th}$ instar larvae was reduced, when they were subjected to the treatment for 6 h. The resistance of the 4$^{th}$ instar larvae to NPV was not reduced when they were subjected to the high temp (37°C) treatment for 6 h, whereas the resistance of the 5$^{th}$ instar larvae was reduced when they were exposed to the treatment for 3 hours.

**Maeda S (1984). A plaque assay and cloning of *Bombyx mori* nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 53:547-548. [Japanese]**

## Vol. 54 1985

**Ito T, Hukuhara T and Akami K (1985). Improved method of detection of polyhedra of a cytoplasmic polyhedrosis recovered from silkworm feces. *J. Sericult. Sci. Jpn.* 54:1-5.**

The occurrence of cytoplasmic polyhedrosis in populations of silkworm larvae can be assessed by detecting polyhedra emitted in the feces with the use of the enzyme linked

immunosorbent assay (ELISA). In an attempt to improve the assay, a sandwhich method was successfully used. Use of purified antibody (IgG fraction) instead of antiserum increased the sensitivity of ELISA on account of the decrease of the non-specific interference. As few as 35.4 polyhedra/well can be detected by the improved method. The minimum time required between the infection of third instar larvae and the recovery and detection of polyhedra from the feces was two days. Dilution end point for the detection was 400 times when distilled water wass used as dilutent and 50 times when feces of healthy larvae were used as diluent.

**Fujiwara T (1985). Microsporidia from silkworm moths in egg-rpduction sericulture. *J. Sericult. Sci. Jpn.* 54:108-111. [Japanese]**

**Fujiwara T, Kagawa T and Andou H (1985). *Nosema bombycis*-like microsporidia isolated from silkworm moths in egg-rpduction sericulture. *J. Sericult. Sci. Jpn.* 54:117-121. [Japanese]**

**Enomoto S, Moriyamma H and Iwanami S (1985). Survival and spread dynamics of *Serratia marcescence* on rearing beds for the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 54:197-201. [Japanese]**

**Iwanami S, Enomoto S and Tomita K (1985). Death of larvae and pupae of the silkworm, *Bombyx mori*, caused by the bacterial contamination of the inner side of the cocoon. *J. Sericult. Sci. Jpn.* 54:278-283.**

Bacteriological examination of the dead larvae and pupae in cocoon collected in Ibaraki Prefecture in 1981 showed that 20-30% of the deaths were caused by septicemia, the proportion being especially high for pupae. The pathogens isolated were *Serratia marcescens*, *Pseudomona* sp., *Proteus* sp. and *Streptococcus faecalis*. Numerous *S. marcescens* bacteria were isolated from the dead larvae and pupae reared in early or late autumn. Mulberry leaves coated with each of the culture of *S. marcescens* and *Streptococcus faecium* bacteria pathogenic to silkworm were fed to silkworms on the $5^{th}$ day of the $5^{th}$ instar and the occurrence of deaths was examined. A high mortality was detected for the pupae in the silkworms fed the leaves inoculated with *S. marcescens* and a high mortality for the larvae of the silkworms fed the leaves inoculated with *S. faecium*. Many of the inoculated bacteria were isolated from the exuvia in the cocoon. This finding suggests that the bacteria adhering to the larvae were introduced into the cocoon and that the exuvia are the source of contamination in the cocoon. The bacteria in the cocoon propagate easily under high temperature and moist conditions hence promoting the contamination in the cocoon. It could be demonstrated that the bleeding pupae in the cocoons contaminated with pathogenic bacteria had died as a result of bacterial infection.

**Tojo A (1985). Enzyme-linked immunosorbant assay for the toxic fragmen of bipyramidal δ-endotoxin produced by *Bacillus thuringiensis kurstaki* strain HD-1. *J. Sericult. Sci. Jpn.* 54:304-309. [English]**

The liberation and decomposition of the toxic principle (p-59), a protein with a molecular weight of 59,000 of *Bacillus thuringiensis kurtaki* strain HD-1 bipyramida δ-endotoxin in the larval gut juice of the silkworm, *Bombyx mori* were investigated by using ELISA and bioassay. The sensitivity limit of the ELISA for the quantification of p-59 was lower than 0.4 ng/ml. When estimated by ELISA, the amount of p-59 antigen produced reached its maximum value within 30 sec after the treatment of δ-endotoxin with gut juice (protease activity: 7.8 U/ml) and this valued retained fro 1 h. After 2-3 h incubation, the antigenic decomposition of p-59 was evident. No change was observed in the toxicity of δ-endotoxin to the silkworm after incubation with gut juice for 1 h, but it decreased by half after incubation for 4 h. When δ-endotoxin was incubated with each of the protease (<0.1 U/ml) from gut juice of the silkworm, the reduction of either p-59 antingen or its toxicity was not observed even after incubation for 4 h. From these results, p-59

seems to be unstable at a high concentration of protease, but stable in diluted one. This suggests that a proper concentration of gut juice protease of the silkworm, ELISA for p-59 is an effective technique for the estimation of the activity of bipyramidal δ-endotoxin toward lepidopteran insect.

**Noguchi Y and Yamaguchi K (1985). Virus susceptibility of regenerated midgut cells by administration of agricultural chemicals in the fall webworm, *Hyphantria cunea*. *J. Sericult. Sci. Jpn.* 54:310-314.**

In the fall webworm larvae, *hyphantria cunea*, administration of sublethal doses of several agricultural chemicals such as PAP, DEP, $CuSO_4$ and formalin resulted in the regeneration of damaged epithelial cells in the midgut of each treated larva. Challenging experiments using BmCPV in these insects however failed to reveal the enhancement of resistance against the infection with this virus.

Previously it was reported that the fall webworm was able to recovery spontaneously from the infection with BmCPV. Furthermore, the insects which had recovered showed a marked resistance against the same virus, upon secondary challenge. The results of the experiments in the fall webworm suggest that the enhancement of the resistance against BmCPV can only be observed when the regeneration of the midgut cells follows the injury caused by the infection with the same virus.

**Ishihara R (1985). Researches in pebrine for the past twenty years. *J. Sericult. Sci. Jpn.* 54:347-353. [Japanese]**

**Seki H (1985). Interstrain difference in the resistance to the infection with densonucleosis virus (Ymanashi isolate) in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 54:445-448.**

Infectivity of the densonucleosis virus (Yamanashi isolate) in silkworm strains was studied by using 34 original strains (Japanese races 19, Chinese races 9, European races 6 and 7 hybrids). Moreover, the mechanism of acquisition of the resistance gene to DNV by N124, a resistant race was analysed. The results obtained were as follows:

The resistant strains included 8 Japanese races *ie*, Aozuku (A), Tanegashima, N122, N122 (futo), N124, N132, N137, N138, 1 Chinese race (Kosetsu) and 2 European races (Ascoli, Gubbio). The other races were susceptible. These results showed the presence of a negative correlation with the resistance to infection to the Ina isolate of DNV.

It was speculated from the results of infectivity in the breeding materials that the resistance gene to DNV preexisted in the progenitors of N124, which had inherited it during the long period of the breeding process.

**Iizuka T (1985). Quantitative analysis of several low molecular antibacterial substances in the digestive juice of 5$^{th}$-instar silkworm larvae. *J. Sericult. Sci. Jpn.* 54:449-452.**

Caffeic acid (CA), protocatechuic acid (PA) and p-hydroxybenzoinc acid (HA), which are low molecular antibacterial substances, had been identified in the digestive juice of silkworm larvae by Koike *et al (1979).* Quantity of CA, which is the main antibacterial substance among the three substances, is considered to play a significant role in the antibacterial activity of silkworms.

In this experiment, the content of CA in samples from 3 days old 5$^{th}$ instar larvae and 5 days old larvae were found to be 250 ppm and 440 ppm respectively. In larvae immediately after molting and 7 days old ones, however, CA content was only 0.6 and 1.3 ppm respectively. In

spite of the small quantities of CA, actual antibacterial activity was much higher than in the samples from 3 days only and 5 days old larvae. This finding suggests that low molecular antibacterial substances did not play an important role in these samples.

**Matsumoto T, Ya-Feng Zhu and Kurisu K (1985). Mixed infection with infectious flacherie virus and bacteria in *Bombyx mori*. *J. Sericult. Sci. Jpn.* 54:453-458.**

Various doses of the infectious flacherie virus (IFV) were injected to gnotobiotic larvae of the silkworm in different instars. Death and arrested development were observed in the infected larvae. IFV susceptibility in the larvae in the early stages of development was $10^3$-$10^6$ times as high as that of larvae in later stages. Ordinary larvae fed on artificial diet or on mulberry leaves were 10-30 times more sensitive to IFV infection than gnotobiotic larvae in each instar.

The susceptibility of the IFV infected larvae increased in presence of *Streptococcus faecalis* and *Serratia marcescens*. The intestine of larvae infected with IFV and these bacteria contained a large number of bacteria in the moulting period. When chloramphenicol was administered to these infected larvae, the bacteria that remained at molting were removed from the intestine and the life of the infected larvae could be prolonged. The bacteria associated with higher virulence in IFV infection were endogeneous *S. faecalis* which is the main component of the intestinal flora of the healthy larvae compared with exogeneous *S. marcescens.*

**Arakawa A and Shimizu S (1985). A dot immunobinding assay for the detection of densonucleosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 54:500-503.**

A dot immunobinding assay (DIBA) for the detection of densonucleosis virus (DNV) of the silkworm, *Bombyx mori* was described. The DIBA (10 ng/ml) was similar to ELISA (6 ng/ml) and bioassay in sensitivity and was 1800, 1000 and 50 times more sensitive than gel immunodiffusion, ring test and latex agglutination, respectively.

**Seki H and Kawase S (1985). Excreation of viruses from the silkworm larvae infected with DNV (Yamanashi isolate) and purification of the viruses from their feces. *J. Sericult. Sci. Jpn.* 54:523-524. [Japanese]**

## Vol. 55 1986

**Matsumoto T, Ya-Feng Zhu, Kurisu K and Akai H (1986). Effects of anti-juvenile hormone on mixed infection of infectious flacherie virus and bacteria in silkworm larvae. *J. Sericult. Sci. Jpn.* 55:1-4.**

Trimolter larvae were induced from aseptically reared larvae fed on an artificial diet by the administration of anti-juvenoid (AJH) in the 3$^{rd}$ instar larval stage. The susceptibility of trimolters to mixed infection of flacherie virus (IFV) and bacteria was determined. When a mixture of IFV ($10^{-2}$ -$10^{-5}$) and *Streptococcus faecalis* ($10^6$) or *Serratia marcescens* ($19^6$) was given to the 4$^{th}$ instar larvae, the mortality of trimolters which had received AJH was about 17-35% compared with that of larvae which had not received AJH. The administration of AJH to the infected larvae prevented death from occurring and the presence of *S. faecalis* or *S. marcescens* in the intestine of the larvae could not be detected. However bacteria accumulated in the intestine of larvae, which AJH had been administered at the 3$^{rd}$ instar and which had been infected with IFV in the presence of *S. faecalis* at the 4$^{th}$ instar. However, the bacteria were not observed in larvae infected with IFV associated with *S. marcescens*.

**Sato R and Watanabe H (1986). Sporogonial sequence in Microsporideae pathogenic to the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:10-16.**

The developmental stages of three Microsporideae; *Nosema* sp. M11, *Nosema* sp. M12, *Pleistophora* sp. were investigated by electron microscopy in relation to their classification into genera. Binary fission of sporonts and single spore formation were observed in sporogonia of *Nosema* sp. M11 and the designation of this Microsporidea as *Nosema* seemed to be appropriate. *Nosema* sp. M12 showed two types of sporogonial sequence. In one sequence, binary fission of sporonts and single spore formation were observed and in another, eight spores were formed in a pansporoblastic membrane. Therefore, this Microsporidea should be reclassified and assigned to the genus *Vairimorpha.* In the sporogonial sequence of *Pleistophora* sp., after formation of multinucleate cells with a triple membrane on the surface, several nuclear divisions and segmentations of the cells occurred, resulting in the formation of spores in a pansporoblastic membrane. Single spore formation without pansporoblastic membrane was observed simultaneously in the host cells. These results suggest that this Microsporidea should be assigned to a new genus.

**Sato R and Watanabe H (1986). Pathways of oral infection in the four Microsporideae in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:17-22.**

Pathways of oral infection with four Microsporidea in the 2nd instar larvae of *Bombyx mori* were reinvestigated. 1. Spores of *Nosema bombycis* extruded their polar filaments in every area of the mid-gut lumen. Sporoplasms and intermediate forms between sporoplasm and schizont of *N. bombycis* were found in the cytoplasm of the midgut epithelium and the circular muscle of the middle part of the midgut 5 and 25 h after spore ingestion. Two days later colonies of schizonts appeared in the cytoplasm of the cells of the mid-gut epithelium, circular muscles, longitudinal muscles and adjacent tissues of the mid-gut. 2. Sporoplasm of *Nosema* sp. M11 were found in the cytoplasm of the mid-gut epithelium 6 h after spore ingestion. Four days later, schizonts of M11 proliferated in the mid-gut epithelium, muscle of the posterior area of the mid-gut, and their adjacent tissues. 3. Spores of *Vairimorpha* sp. M12 germinated in every area of the mid-gut lumen and their sporoplasms were observed in the cytoplasm of the mid-gut epithelium and middle part of the mid-gut. Two days later, schizonts of M12 were seen in the same tissues and sometimes in the adjacent tissues. 4. Spore of *Pleistophora* sp. extruded their polar filaments in every area of the mid-gut lumen. Based on these observations, the pathways of oral infection with these four Microsporideae were discussed.

**Sato R and Watanabe H (1986). *In vitro* infection with spores of Microsporideae contained in diseased silkworm. *J. Sericult. Sci. Jpn.* 55:28-32.**

The homogenates from the larval tissues of the silkworm, *Bombyx mori* infected with *Nosema* sp. M11 or *Pleistophora* sp. were infective to *Antheraea eucalypti* cells *in vitro*. The homogenates placed in Grace's insect medium maintained their infectivity to the *A. eucalypti* cells for at least 12 h. When the homogenates were added to the culture, infection of these two Microsporideae and the rates of infection increased with time up to three hours. When observed under an electron microscope, the infectious bodies of the Microsporideae in culture cells showed the same structural features as those of their sporoplasms. Among the fractions obtained through isotonic Percoll density equilibrium centrifugation, only sporoblast and spore fractions of *Nosema* sp. Maa and spore fraction of *Pleistophora* sp. were able to infect *A. eucalypti*. The very few spores of *Nosema* sp. M11 fractionated by Percoll centrifugation, and the spores contained in the homogenate were found to extrude their polar filaments and sporoplasms in the Grace's medium at 26°C within a day. These results indicate that the infectivity of homogenates to *A. eucalypti* cells *in vitro* resulted from autogermination of spores in Grace's medium.

**Watanabe H, Kawase S, Shimizu T and Seki H (1986). Difference in serological characteristics of densonucleosis viruses in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:75-76. [Japanese]**

**Shimizu S and Arakawa A (1986). Latex agglutination test for the detection of the cytoplasmic polyhedrosis virus and the densonucleosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:153-157.**

Agglutination tests using antibody-sensitized latex particles were developed for the specific detection of the cytoplasmic polyhedrosis virus (CPV) and densonucleosis virus (DNV) of the silkworm, *Bombyx mori*. With this test, 0.75 µg/ml of CPV or 0.5 µg/ml of DNV protein could be detected. The sensitivity of latex agglutination test was 20-30 or 5-20 times higher than that of immunoduffusion or ring test, and was 100-200 times lower than that of ELISA, respectively. The tests completed within 5 min. Extracts from CPV or DNV infected silkworm larvae agglutinated latex particles specifically, while there was no agglutination by extracts from normal silkworm larvae. The results showed that the sensitivity and simplicity of this technique for detection of CPV and DNV were greater than those of conventional serological techniques such as the immunodiffusion test.

**Hukuhara T, Iso M and Aba H (1986). The effects of serotonin on the midgut motility and virus resistance in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:158-162.**

The motility of silkworm larval midgut was enhanced by serotinin and its precursor, 5-hydroxy-tryptophan. The frequency of midgut contractions gradually increased and reached a plateau (26-30% higher than the normal value) 9n 1-2 h following the initiation of peroral chemical administration. When it was discontinued in 6 h, the midgut motility maintained the same high level for 2 h, then declined and reached the normal level in 4-5 h. The chemical administration increased the rate of food transport in the midgut, shortened the stay of midgut content and increased the resistance of silkworm larvae to orally administered cytoplasmic polyhedrosis virus.

**Eguchi R, Furuta Y and Ninaki O (1986). Dominant nonsusceptibility to densonucleosis virus in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:177-178. [English]**

Densonucleosis virus (DNV) of the silkworm, *Bombyx mori*, has been classified into several types based on chemical and pathological characteristics, *eg*, Ina virus, Saku virus, Yamanashi virus, etc. The larvae of certain silkworm strains are highly susceptible to the Ina virus and non-susceptible to the Yamanashi virus, while those of other strains are susceptible to the Yamanashi virus and non susceptible to the Ina virus. It is assumed that the non-susceptibility to the virus is controlled by a recessive gene respectively. We observed a strain in which the non-susceptibility to the Ina virus was considered to be controlled by a dominant gene based on gene analysis of the recessive non-susceptibility of the strain to the Ina virus. We report here on the mode of dominant non-susceptibility to the Ina virus in the silkworm.

**Miyajima S and Kawase S (1986). Inhibitory effects of several guanidine salts and derivatives on the incidence of infectious flacherie in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:202-204.**

In previous papers, we reported that guanidine hydrochloride has an inhibitory effect on the incidence of infectious flacherie in the silkworm, *Bombyx mori*. In this paper, several salts of guanidine and derivatives were tested for their inhibitory effects on the disease. Of these chemicals, guanidine carbonate, guanidine nitrate, guanidine phosphate and guanidine sulfamate reduced the incidence of infectious flacherie in the same way as guanidine hydrochloride did. On the other hand, sulfaguanidine, dedecylguanidine acetate and octylguanidine acetate, which do not dissociate into guanidine radicals, failed to reduce it. When guanidine carbonate was heated at 200°C for 1 h to decompose the guanidile radicals, it lost its inhibitory effect on the virus. From these results, it is suggested that the antiviral activity of several guanidine salts on the infectious flacherie virus is associated with the guanidile radical.

**Miyajima S, Washida S, Kurashima H and Kawase S (1986). Effects of guanidine administration for the control of infectious flacherie of the silkworm on the quality of the cocoon. *J. Sericult. Sci. Jpn.* 55:216-219.**

Several properties of the cocoon and raw silk produced by the larvae reared on an artificial diet or mulberry leaves to which 0.01% guanidine hydrochloride had been added to control the incidence of infectious flacherie, were investigated. No differences in the properties of the cocoon and raw silk, including the length and weight of the cocoon filament, raw silk percentage, reelability percentage, etc., were observed between the control and the guanidine treated larvae. Since this chemical is fairly cheap, it could be used as an antiviral or prevention agent against infectious flacherie of the silkworm.

**Kawakami K and Shimane T (1986). Microbial control of the yellow-spotted longocorn beetle, *Psacothea hilaris* PASCOE (Coleoptera; Cerabycidae), by an entomogenous fungus, *Beauveria tenella*. *J. Sericult. Sci. Jpn.* 55:227-234.**

An entomogenous fungus, *Beauveria tenella*, was used to control of the most serious insect pest *Psacothea hilaris*, in mulberry plantation of eastern part of Japan. Mass production of conidia of *B. tenella* were carried out with several kinds of organic solid materials, and wheat bran as basic culture substrate was most effective for conidial production. Approximately $10^9$ conidia/g of dried wheat bran were produced after 15 days at 25°C. A two step culture technique for mass production of *B. tenella* conidia showed good effect to shortening the cultural period and to reduce the contamination by other microorganisms. First *B. tenella* was produced as hyphal bodies by shaken culture, and they were surface cultured for 10 days at 25°C on wheat bran in trays for sporulation. In the file cage tests, 1-3 kg/acre of wheat bran with *B. tenella* conidia were applied only one time on the field surface of mulberry plantation after the release of adult pest insects. Cumulative mortalities of adult insects for 30 days averaged 60-90% respectively. The above results suggest that the regulated application of *B. tenella* probably has practical value as a microbial insecticide.

**Miyajima S and Washida S (1986). Inhibitory effect of inactivated polyhedra added to an artificial diet on the incidence of cytoplasmic polyhedrosis in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:297-300.**

Resistance to the cytoplasmic polyhedrosis virus (CPV) of the silkworm, *Bombyx mori* reared on artificial diet containing inactivated polyhedra was examined. When the silkworm larvae reared on an artificial diet containing the cytoplasmic polyhedra inactivated at 85°C for 15 min. were perorally inoculated with CPV, the incidence of cytoplasmic polyhedrosis was remarkably reduced. However, the inhibitory effect on the virus could not be observed when the larvae reared on this diet were infected by intrahemocoelic inoculation. Consequently, the interaction between heat inactivated cytoplasmic polyhedra and the active ones were recognized only when the larvae were subjected to peroral inoculation of the active virus.

**Miyajima S (1986). Synergism in the incidence of cytoplasmic polyhedrosis by co-infection of species of bacteria in the silkworm, *Bombyx mori*, reared on artificial diets. *J. Sericult. Sci. Jpn.* 55:301-304.**

The larvae were reared on an artificial diet wetted with an antibiotic (Streptomycin or penicillin) and a suspension of *Serratia marcescens*, which is a nonpathogenic bacterium to silkworm, at a high concentration (McFahland No.3) from the 1st instar onwards. Threafter the larvae were inoculated *per os* with cytoplasmic polyhedrosis virus at the 3rd instar just after ecdysis. The incidence rate of the cytoplasmic polyhedrosis increased by co-infection with *Serratia marcescens* at a high concentration, but the rate decreased by addition of antibiotics. From these results, it was demonstrated that co-infection with a large amount of non-pathogenic

bacteria led to the increase of the incidence of cytoplasmic polyhedrosis. The synergistic effect of the bacteria was lost by addition of antibiotics.

**Kurisu K (1986). Simplified calculation of the operating characteristics in the pebrine inspection of the grouping mother moths method. *J. Sericult. Sci. Jpn.* 55:351-352. [Japanese]**

**Arakawa A and Shimizu S (1986). Effect of some factors in the latex agglutination test for the diagnosis of virus infectious larvae in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 55:384-387.**

The effect of IgG concentration, diameter of latex particles and their density on the latex agglutination test was examined in order to evaluate the test for the detection of infectious flacherie virus (IV), densonucleosis virus (DNV) and cytoplasmic polyhedrosis virus (CPV) in diseased silkworm larvae. It was found that a higher concentration of IgG for sensitization resulted in a higher degree of agglutination. However, when the concentration of IgG was too high, the agglutination process was impaired. Optimum diameter of the latex particles for the detection of IFV and DNV was 0.65 μm or 1.00 while that for the detection of CPV was 0.45 μm. The variations in the diameter of the latex particles also affected the speed of the reaction and the degree of agglutination. The results showed that by adjusting these factors the same degree of agglutination could be obtained in different IgG sensitized latex preparations.

**Matsuno M and Nishina S (1986). Conditions of conidial germination in the fungus *Diaporthe nomurai* Hara. *J. Sericult. Sci. Jpn.* 55:415-420.**

The percentage of conidial germination of *Diaporthe nomurai* HARA was low in distilled water. However, in potaro dextrose medium or at an appropriate concentration of the mulberry tree extract, a high rate of germination was obtained. Conidia of the fungus could germinate in the temperature ranges of 15°C to 35°C. The percentage of conidial germination was only 19% and 53% or a relative humidity of 93% and 98% respectively. The optimum temperature for the germination was approximately 27°C and a relative humidity of nearly 100% was required. The light conditions or darkness did not affect the germination.

In mulberry lenticles, the conidia of the fungus were able to germinate in the temperature range of 10°C to 35°C, the range of 20°c and 25°c being optimum. At a relative humidity of more than 93%, the percentage of conidial germination ranged from 53% to 71%, but it decreased markedly at a relative humidity between 32.5% and 86%. The percentage of germination of the conidia did not increase under the optimum conditions after incubation of the conidia at a high temperature or low relative humidity for 5 days.

**Seki H (1986). Epizootiology of silkworm densonucleosis caused by the Yamanashi isolate of the virus. *J. Sericult. Sci. Jpn.* 55:421-427.**

Densonucleosis caused by the Yamanashi isolate (Y-DNV) of the virus occurred rarely in the sericultural farms and only in restricted areas of Yamanashi prefecture. This disease did not appear to spread to other regions. Spread of the pathogen by secondary infection was negligible in the rearing environment, as this disease was chronic. Larvae infected with Y-DNV after the 4$^{th}$ instar did not die in the 5$^{th}$ instar. Of the six lepidopterous insects tested, only *Bombyx mandarina* was infected with Y-DNV. Since the population of this insect is seldom high in the mulberry plantations, the probability of cross infection from infected *B. mandarina* to the silkworm appears to be low. It was concluded that the virus which persisted in the rearing environment gave rise to the year-to-year infection cycle of the silkworm.

**Shimisu S and Arakawa A (1986). Prevention of non-specific positive reaction on the latex agglutination test for diagnosis of virus infectious silkworm. *J. Sericult. Sci. Jpn.* 55:439-440. [Japanese]**

**Shimisu S (1986). A method for the isolation of amino acid-requiring mutants in *Beauveria bassiana. J. Sericult. Sci. Jpn.* 55:472-476.**

A method for the isolation of amino acid requiring mutants of *Beauveria bassiana* by filtration enrichment and replica technique using velvet was developed. By filtration, an average enrichment of 15.2 fold was achieved during isolation of 18 amino acid requiring mutants. By using sorbose agar medium, replica technique used in bacteria was possible and facilitated the isolation of amino acid requiring mutants in *B. bassiana.*

**Shimisu S (1986). Properties of protoplasts of *Beauveria bassiana. J. Sericult. Sci. Jpn.* 55:510-517.**

A high yield of protoplasts from young mycelia of *Beauveria bassiana* was obtained by treatment with Zymolyse 20-T. Among the protplasts, 27.5% laching a nucleus, 52.3% of them containing a single nucleus, and the other protoplasts contained two or more nuclei. Mycelia and protoplasts of *B. bassiana* were treated with Calcoflur white (CAW) and FITC labeled lectins. Mycelia and protoplasts reacted with Concanavalin A (Con A), but the former reacted with CAW, Soybean agglutinin (SBA) and Peanur agglutinin (PNA). These results indicate that either chitin or cellulose may be the major structural components of the cell wall of *B. bassiana*. The regenerating protoplasts remained spherical in shape for a long time (usually 6-8 h) and they could not be distinguished from non regenerating protoplasts under a phase contrast microscope. However, cell wall regeneration can be observed under a fluorescence microscope after staining with CAW, SBA or PNA.

**Kurisu K, Imanishi H and Hamazaki M (1986). Group-in-group sequential pebrine inspection table of the grouping mother moths method in the silkworm. *J. Sericult. Sci. Jpn.* 55:525-526. [Japanese]**

**Eguchi M and Yoshi S (1986). Inhibition of protease from muscardine fungus by the inhibitor in the silkworm haemolymph and tissues. *J. Sericult. Sci. Jpn.* 55:531-532. [Japanese]**

## Vol. 56 1987

**Arakawa A and Shimizu S (1987). Quantitative assay of densonucleosis virus of the silkworm, *Bombyx mori*, by the latex agglutination test. *J. Sericult. Sci. Jpn.* 56:29-32.**

Latex agglutination test as a quantitative assay for densonucleosis virus of the silkworm *Bombyx mori* was developed. The effects of varying proportions of virus, antibody sensitized latex, shaking and incubation time were investigated and optimum conditions for the reaction were determined. Addition of larval extracts did not inhibit the virus-antibody reaction in this assay system. The amount of virus in the infected larvae estimated by the latex agglutination test was proportional to that estimated by the single radial immunodiffusion test.

**Utsumi S, Akai A and Nomura S (1987). Anti-streptococcal activity of digestive juice in various strains of silkworm, *Bombyx mori. J. Sericult. Sci. Jpn.* 56:109-115.**

In order to elucidate the genetic control of the anti-streptococcal activity (ASA) in the digestive juice (DJ) of the silkworm, *Bombyx mori*, the ASA was investigated on various strains and their hybrids which were reared on mulberry leaves until the 3$^{rd}$ day of 5$^{th}$ instar. The DJ was

fractionated by the step wise addition of saturated ammonium sulfate at 10% intervals and the ASA in terms of $ASA_{90}$, which indicates the dilution times to attain $ED_{90}$ in the inhibition on the growth of test bacterium, was assayed *in vitro* for each fraction. In each strain so far investigated ASA was detected between 20-80% saturation of ammonium sulfate and there were two peaks of ASA, *ie.*, in 30-40% and 50-60% or 60-70% saturations. The total $ASA_{90}$ was distributed in the range between 300 and 600 and the value of the hybrids was higher than those in their parents, which seemed to indicate heterosis.

When the protein fraction prepared by the precipitation of DJ with ammonium sulfate at 80% saturation was applied to Sepharose 6B, ASA was eluted as three peaks, which were named as A, B and C. The elution profile indicated incomplete separation between A and B but the clear separation between A or B and C. Strain $H_4$ had higher peaks in A and B compared with one of Kojiki and Daizo, both of which are considered to be more classical strains of the silkworms. The ASA of the hybrid, Kojiki x Daizo showed approximate value to the higher of their parents. These results suggest that the anti-streptococcal activity in the digestive juice of the silkworm, *Bombyx mori* might be controlled genetically.

**Hayasaka S and Ayuzawa C (1987). Diagnosis of microsporidian, *Nosema bombycis* and closely related species by antibody sensitized latex. *J. Sericult. Sci. Jpn.* 56:169-170. [English]**

In the present report, the authors extended the application of the antibody sensitized latex agglutination test to the detection of microsporidian spores for the first time and also observed that not only spore aggregation but also latex particle attachment on the spore surface is highly efficient for detecting microsporidian spores.

**Kobayashi M and Tanaka K (1987). Histopathological study on the replication specificity of the nucleopolyhedrosis virus in the pericardial cells of *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 56:196-201.**

Pericardial cells of insects are known to incorporate foreign matter. The replication process of nucleopolyhedrosis virus in *Bombyx mori* L., was characterized by comparing the morphology of the pericardial cells and tracheal cells which are susceptible to determine the infective specificity in tissues and to indentify the cellular defense mechanisms against the nucleopolyhedrosis virus. The pattern of virus multiplication was similar in the pericardial cells and in the sensitive cells. However, the following differences were noted. (1) The viral multiplication in the pericardial cells was somewhat delayed, due to the accumulation of proteinic granules in the pericardial cells. (2) In the pericardial cells, the virogenic stroma was immature with a low production of virus rods (3) In the gaps of the basement membrane of the pericardial cells virus rods accumulated at the initial and final stages of infection (4) In the pericardial cells, the polyhedra were abnormal in shape and scanty in number, containing no or few virus rods.

**Ichida M, Ayuzawa C and Akai H (1987). Effect of treatment with antijuvenoid on the resistance to the infection with virus in *Bombyx mori*. *J. Sericult. Sci. Jpn.* 56:216-219. [Japanese]**

**Yanagita T (1987). Studies on oral infection of larvae of the silkworm, *Bombyx mori*, with *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 56:279-284.**

Infection of the silkworm larvae by oral inoculation of conidia of *Beauveria bassiana* at the time of feeding was studied. Germination of the fungus conidia and their hyphal growth could be observed in the digestive juice of the silkworm larvae used as medium. Dipping in 70% ethanol solution of the larvae subjected to oral inoculation of the fungi was effective in disinfecting the

body surface of the larvae. After oral inoculation, conidia on the body surface of the larvae were killed by dipping in 70% ethanol solution. 2nd and 3rd instar larvae subjected to the oral inoculation of conidia of *Beauveria bassiana* died from fungus disease. The number of larvae which died due to oral inoculation of the fungi was lower than in the case of cutaneous inoculation. Fungus disease occurred chronically in the case of oral inoculation and the infected larvae died mainly during the period of molting. When 5th instar larvae were subjected to the oral inoculation of the fungi, the silkworms died at the stage when pupae was incompletely exuviated and did not spin a cocoon. *Beauveria bassiana* was recovered from the alimentary canal of a large number of silkworms.

**Yanagita T and Iwashita Y (1987). Histological observation of larvae of the silkworm, *Bombyx mori*, orally infected with *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 56:285-291.**

To evaluate the possibility of oral infection of larvae of *Bombyx mori* with *Beauveria bassiana*, the pathological changes in the alimentary canal of the silkworm larvae were observed by light and electron microscopy after oral inoculation of conidia to the silkworms at the time of feeding. Oral infection with *Beauveria bassiana* was investigated by histological observation. After oral inoculation of conidia of *Beauveria bassiana* to the 2nd instar larvae, the germination of the conidia was observed in the alimentary canal. After hyphal propagation in the alimentary canal, the apical part of the columnar cells become swollen and the columnar cells were destroyed.

When the hyphae penetrated into the goblet cavity, the microvilli of the goblet cells changed to the lamellar state, and the goblet cells were destroyed. Due to the destruction of the midgut hyphae penetrated from the intercellular spaces into the midgut tissues. Direct hyphal invasion was not detected in the midgut tissues. Based on these observations, it was considered that oral infection after inoculation of *Beauveria bassiana* occurred due to hyphal penetration into the body cavity from the intercellular space made in the midgut tissues, after their destruction by the toxins or substances secreted from the hyphae and was not caused by direct hyphal invasion into the midgut tissues.

**Nakagaki M, Takei R and Nagashima E (1987). Improved method of purification of the infectious flacherie virus the *Bombyx mori* densonucleosis virus. *J. Sericult. Sci. Jpn.* 56:338-342.**

Concentration of ammonium sulfate required to precipitate the infectious flacherie virus (IFV) and DNV was determined. IFV and DNV in precipitate gave the maximum yield at 40% and 55% saturation, respectively. Separation of IFV and DNV with a Hitachi RPV50T vertical rotor instead of a conventional swinging bucket rotor was attempted. A 3 ml virus sample of IFV and DNV was layered on the top of a 36 ml sucrose gradient (10 to 40%) and centrifuged at 85,000 g for 30 min. in the RPV 50T rotor. The two viruses could be separated successfully. The vertical rotor shortened the separation time and afforded a high level of purification.

**Kawase S and Seki H (1987). Elimination of contaminating infectious flacherie virus from the purified preparation of BmDNV. *J. Sericult. Sci. Jpn.* 56:355-356. [Japanese]**

**Iizuka T and Goto C (1987). Toxic effect of crystal protein from *Bacillus thuringiensis* on *Bombyx mori* and *Mamestra brassicae*. *J. Sericult. Sci. Jpn.* 56:379-384. [English]**

Toxicity of crystals isolated from several strains of *Bacillus thuringiensis* were tested on larvae of the silkworm, *Bombyx mori* and the cabbage moth *Mamestra brassicae*. Values of the $LD_{50}$'s (50% lethal dose) of the crystals for the 5th instar larvae of the silkworm were as follows:

0.01μg for subsp. *sotto*, 0.1 μg for *kurstaki* HD-1, 30.0 μg for *kurstaki* HD-73 and 0.2 μg for *wuhanensis*. On the other hand, the values of the $LD_{50}$'s of the crystals for the 5th instar larvae

of the cabbage moth were 46 µg for *sotto*, 33 µg for HD-1, 23 µg for *wuhanensis* and 21 µg for *aizawai*. However, the crystals from HD-73 were not toxic to the cabbage moth larvae at the level of 1,000 µg/ larva. The crystals of HD-73 released a 135 kDa protein when added to the gut juice proteinase from silkworm larae, whereas no 135 kDa protein was released when the crystals were added to the gut juice proteinase from *M. brassicase*.

**Abe Y (1987). *In vitro* encystations of *Leptomana* sp., an entomopathogenic flagellate of the silkworm, *Bombyx mori* L. *J. Sericult. Sci. Jpn.* 56:428-430.**

Formation of cyst membrane *Leptomonas* sp. was observed by histochemical methods and *in vitro* culture. An acid mucopolysaccharide and sulfuric protein were detected in the cyst membrane by histo chemistry. The encystations was induced successfully when hyaluronic acid (or both N-acetyl-D-glucosamine and D. glucuronic acid) and bovine albumin fraction V were added to modified Bm22 or GRACE's insect cell culture medium.

**Han MS and Watanabe H (1987). Immunoperoxidase-staining methods for discrimination of Microsporidian spores in the pebrine inspection of silkworm mother moths. *J. Sericult. Sci. Jpn.* 56:431-435.**

In the pebrine inspection of silkworm mother moths, the presence of Microsporidian spores similar to those of *Nosema bombycis* in size and shape hampers the discrimination of *N. bombycis* spores from those of other microsporidia. In order to solve this problem, immunoperoxidase staining methods that enable to discriminate Microsporidian spores in the inspection preparation of silkworm mother moths dried at 70°C for 2 h and then macerated in 2% KOH were investigated. Two practical methods, the indirect immunoperoxidase (IIP) method and the peroxidase antiperoxidase complex (PAP) method, which enable to discriminate spores easily by specific staining with diaminobenzidine-4HCl as a chromogenic substrate, were developed and their procedures were desribed in the present paper.

**Abe H, Watanabe H and Eguchi R (1987). General relationship between non susceptibilities of the silkworm, *Bombyx mori* to two densonucleosis virus. *J. Sericult. Sci. Jpn.* 56:443-444. [Japanese].**

## Vol. 57 1988

**Utsumi S and Azumai Y (1988). Population density and development of antibiotic resistance of *Streptococcus faecalis* and the related in sericulture environments. *J. Sericult. Sci. Jpn.* 57:8-16.**

To analyse the development of an antibiotic resistance of *Steptococcus faecalis* in seicultural environment, we investigated the specimens collected from rearing farms of the silkworm in Shiga and Kyoto prefectures for the population density and the resistant ratios of these bacteria against antibiotics. In dusts from sericultural environment, $8.0 \times 10^4$ viable cells per g of dust were counted before rearing of silkworm and after rearing the number of viable cells was increased to $7.8 \times 10^6$. Within the intestine of the silkworm larvae, $6.1 \times 10^8$ viable cells per larva were counted in the diseased larvae and about $1 \times 10^4$ cells per larva from from healthy $5^{th}$ instar larva. Ratio of antibiotic resistance against tetracycline (Tc), chloramphenicol (Cm) and Streptomycin (Sm) in the isolates was determined. In many cases, the ratios were greater than 50% of Tc resistant strains (Tc) and the ratio of $Cm^r$ and $Sm^r$ followed. In dusts the ratio has changed about 2.5 times before and after raring of the silkworm but it waas not almost changed in the intestine of sislkworm larvae regardless from the diseased or healthy. By the cross brush method, various multi-resistant strains were found from isolates with Tc, Cm, Sm, Kanyamycin (Km), and Ampicillin (Am). The higher resistants were also obtained with Sm in 4,000 µg/ml,

Km in 200 µg/ml, and Tc in 100 µg/ml. Additionally, a resistant strain by 10 times resistant compared with standard strain was obtained against ant-*Streptococcus* protein which exists in the digestive juice of the silkworm larvae.

**Hayashiya K, Sakaguchi H and Utsumi S (1988). Antibacterial properties of D(-)-2,3-diaminopropionic acid contained in digestive juice of the silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 57:57-61.**

The Digestive juice of silkworm larvae exhibits strong antibacterial properties. D(-)-2, 3-diaminopropionic acid (D-DAPA) is contained in the digestive juice (DJ) (Wada and Toyota) of the silkworm. To determine the relationship between the antibacterial action and the presence of D-DAPA, the antibacterial action of D-DAPA in several species of bacteria was assayed *in vitro*. The concentrations of D-DAPA in the DJ were 1.372 µ moles per ml DJ in larvae fed on mulberry leaves and 0.724 µ moles per ml DJ in larvae fed on an artificial diet immediately after the 4$^{th}$ ecdysis, respectively. Inhibitory activity of D-DPA on the growth of the bacteria was demonstrated in the cases of *Streptococcus faecalis-S. faecium* intermediate IFO 12368, *Bacillus thuringiensis* subsp. *Thuringiensis* and *Serratia marcescens* but not in the cases of *Bacillus cereus* IFO 3001, *Pseudomonas aeruginosa* IFO 3445, *Staphylococcus aureus* IFO 3060 *AND* Escherichia coli K12 strains. Concentration of D-DAPA required for the complete inhibition on the growth of *S. faecalis-S. faecium* intermediate was 10mM, and for partial inhibition in 5mM. Based on these results, it is evident that the antibacterial action in the intestine of the silkworm can not be attributed to D-DAPA alone. D-DAPA seemed to be one of the antibacterial substances present in the digestive tract of the silkworm larvae.

**Abe Y (1988). Insect cell lines promoting the encystation of *Leptomonas* sp., a trypanosmatid flagellate pathogenic to the silkworm, *Bombyx mori* L.*J. Sericult. Sci. Jpn.* 57:79-80. [English]**

It had been suggestd that hyaluronic acid and albumin may be required during the encystation of *Leptomonas* sp., a flagellate pathogen of the silkworm (Abe, 1987). When this flagellate was raised with insect culture cells, the rate of encystation increased compared to the case when the flagellate alone was raised in the medium used for the culture of insect cells.

**Shimizu S and Kazuhiko K (1988). Isolation of protoplasts from several entomopathogenic fungi. *J. Sericult. Sci. Jpn.* 57:81-82. [Japanese]**

**Tomita K and Iwashita Y (1988). Infectivity of *Enterobacter* sp. isolated from *Spilosoma imparilis* to larvae of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 57:133-139.**

The pathogenicity of *Entrobacter* sp. bacteria inoculated to the silkworm larvae was analysed in relation in relation to the stage of growth of the larvae and the temperature during rearing. Observations were also made on the infectivity ratio and multiplication in the cytoplasm of the larvae midgut cells of the bacteria inoculated together with cytoplasmic polyhedrosis virus (CPV). The bacteria did not exhibit any pathogenicity to the 1$^{st}$ to 3$^{rd}$ instar larvae (which were reared on mulberry leaves), while a weak pathogenicity was recognised in the 4$^{th}$ and 5$^{th}$ instar larvae.

When the inoculation was performed at a low temperature (5°C, 24 h) immediately after ecdysis, the bacteria grew well in the 2$^{nd}$ and 3$^{rd}$ instar larvae. In particular a high infectivity ratio was recorded in the 4$^{th}$ and 5$^{th}$ instar larvae. Additionally, when bacteria were inoculated to larvae reared on an artificial diet, a higher pathogenicity ratio was found in the larvae in older instars than in those in younger instars. When a filtered culture solution of the bacteria was administered per os, no larvae became infected. Therefore, it was considered that the bacteria did

not produce any toxins. When the bacteria were added after the inoculation of CPV, the infectivity ratio was higher than that obtained by the inoculation of the bacteria alone.

Electron microscopic observations showed that both CPV and the bacteria multiplied in the midgut cell cytoplam of the larvae and that the bacteria penetrated and multiplied in the virogenic stroma of CPV.

**Abe Y and Kawarabata T (1988). On the microsporidian isolated from the cabbage worm, *Pieris rapae crucivora*. *J. Sericult. Sci. Jpn.* 57:147-150.**

Biological and serological studies were carried out on four microsporidian isolates derived from *Pieris rapae crucivora* comparing with isolated derived from the silkworm, *Bombyx mori*. From biological studies, four isolates of *P. rapae crucivora* were identified as *Nosema bombycis* (one isolate), *Nosema mesnili* (two isolates) and *Pleistophora schubergi* (one isolate). *N. bombycis* (NB-Bm-SES-Std) of *Bombyx mori* and *N. bombycis* (NB-Prc-SES-H7901) of *P. rapae crucivora* showed the homologus serological reaction, while *N. mesnili* (NM-Prc-SES-H7901 and A8301) and *Nosema* sp. (M11) of *B. mori* showed the homologus reaction.

**Utsumi S and Koizumi M (1988). Analysis on the *in vivo* activity of the anti *Streptococcus* protein contained in the digestive juice of silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 57:171-178.**

In the *in vivo* action of anti-*Streptococcus* protein (ASP) contained in the digestive juice (DJ) of silkworm larvae, *Bombyx mori*, was studied. DJ tested was collected after dissection of larvae which had fed and of frozen larvae; anti-*Streptococcus* activity (ASA) in the fraction of saturated precipitates with 80% ammonium sulphate prepared from the DJ of larvae that had fed was stronger than that from starved larvae. ASA in the fraction of the DJ from the larvae that had fed was present of higher dilutions such as 500 and 600, but was absent of lower dilution such as 50 to 200 times. On the other hand, ASA in the fraction of the DJ from starved larvae was present of lower dilutions and was absent after freeze preservation for 120 days, whereas in the fraction of DJ from larvae which had fed, ASA withstood a longer preservation. In the G75-A fraction obtained through a Sephadex G-75 column from the ammonium sulfate precipitate, ASA was present and was inhibited by the addition of other fractions such as G75-B and G75-C, containing protein substances. Based on these results, it is considered that ASP may be associated with various protein substances and that denaturation is prevented or the activity is controlled by the other prtein substances in the intestine.

**Mike A, Ohwaki M and Fukada T (1988). Preparation of monoclonal antibodies to the spores of *Nosema bombycis*, M11 and M12. *J. Sericult. Sci. Jpn.* 57:189-195.**

Splenic lymphocytes from BALB/c mice immunized with spores of *Nosema bombycis* (Nb), *a Nosema* sp. M11 and a *Vairimorpha* sp. M12 were fused with NS-1 mouse myeloma cells to obtain hybridomas-secreting antibodies against their spore specific antigens. Three monoclonal antibodies were established, N321, E324 and T240 specifically bound to the spres of Nb, M11 and M12 respectively. Inhibition ELISA studies also showed that the binding of N321, E324 and T240 to the corresponding spres were inhibited by the homogenate of Nb, M11 and M12 infected silkmoth respectively. These anti Nb, M11 and M12 specific monoclonal antibodies should prove to be of great value as diagnostic and reseach reagnts.

**Abe Y (1988). *Nosema mesnili* isolated from the fall web worm *Hyphantria cunea*. *J. Sericult. Sci. Jpn.* 57:200-202.**

Pathogenicity, mode of multiplication and serological properties were studied in a microsporidium isolated from the fall webworm, *Hyphantria cunea*. This microsporidium was

infectious to the early stage larvae of the fall webworm, the silkwrom *Bombyx mori* and the cotton leaf worm, *Spodoptera litura*. However, the number of spores formed in the fall webworm larvae and in the cotton leaf worm was lower than that formed in the silkworm larvae. In the cotton leaf worm, sporulation was observed mainly in nervous tissues, indicating that the paralysis of the infected larvae was due to the damage of the nervous system associated with the infection. The spores of the microsporidium as well as the spores of *N. mesnili* were agglutinated by the anit-*N. mesnili* spore antiserum.

**Watanabe Y, Yoshihara T and Inoue H (1988). Infection of a *Bombyx* cell line adapted to different media with a nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 57:227-231.**

The effect of the medium composition on the infection of a *Bombyx mori* cell line (SES-BoMo-15A) with a *B. mori* nuclear polyhedrosis virus was examined. When the cells were adapted to the MGM-443 (10% FBS) medium and the MM+3% FBS medium after culture in the MGM-448 (10% FBS) medium, the cells cultured in the MM+3% FBS medium seldom formed polyhedra compared with those cultured in the MGM-443 (10% FBS) and MGM-448 (10% FBS) media after the virus inoculation. However, fluorescent antibody staining against the virus and polyhedral proteins revealed the presence of antigen in the cells inoculated in the MM+3% FBS medium. When the cells adapted to the MM+3% FBS medium were directly transferred to the MGM-443 (3% FBS), MGM-443 (10% FBS), MM+3% FBS and MM+10% FBS media and inoculated with virus, polyhedra were formed in both MGM-443 media and plyhedra formation was more conspicuous in the medium containing 10% FBS than in the 3% FBS medium. However, very few cells in both MM media showed the formation of polyhedra. These results suggest that the MM medium is deficient in the nutrients required for the formation of polyhedra.

**Ichida M (1988). Susceptibility of the trimolter larvae of the silkworm, *Bombyx mori*, induced by an anti-juvenoid, to the infection with *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 57:233-234. [Japanese]**

**Kanke E and Couch EF (1988). Cell number in the midgut of the silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 57:237-238. [English]**

It is well known that the midgut of the silkworm larva contains columnar cells, goblet cells and regenerative cells. Investigators who have examined this tissue hostologically have noted that the number of columnar cells differs from that of the goblet cells. Therefore, a study on the relative frequency of these cells in the midgut might prove useful as a means of clarifying the differene between the columnar cell and the goblet cell. In the present investigation we have undertaken a numerical study as a first step in an attempt to throw light on the physiological roles that these two types of cells play in digestion and absorption.

**Taniai K and Inoue H (1988). Analysis of structural proteins of the nuclear polyhedrosis viruses of *Bombyx mori*, by Western-blotting. *J. Sericult. Sci. Jpn.* 57:265-269.**

The structural proteins of the nuclear polyhedrosis virus of *Bombyx mori* were analysed by SDS-PAGE and Western-blotting. Twenty-one polypeptide bands including polyhedrin were observed by staining with Coomassie brilliant blue, when the alkali dissolved polyhedra were run in SDS-PAGE. In th Western blotting of SDS-PAGE gels, eleven bands reacted with IgG against occluded virus. Polyhedrin band did not react with this anti-NPV-IgG. Among these eleven bands, two polypeptide bands of adjacent 25 K molecular weight were also detected in the hemolymph from deseased larvae, and these two bands of adjacent 25 K were assumed to be common polypeptide of both occluded and non occluded viruses.

**Tomita K and Iwashita Y (1988). Infectivity of *Enterobacter* sp. isolated from *Spilosoma imparilis* to larvae of some insects by intrahemocoelic injection. *J. Sericult. Sci. Jpn.* 57:470-474.**

*Enterobacter* sp. was inoculated with intrahemocoelic injection to larvae of 7 species of insects. Of 7 species, *Bombyx mori*, *Malacosoma neustria testacea*, *Dictyoploca japonica*, *Spilosoma subcarnea* and *Bombyx mandarina* were infected, but *Glyphodes pyloalis* and *Psacothea hilaris* were not infected. The difference in susceptibility to bacterial injection was not found between the silkworm larvae reared with mulberry leaves and with artificaial diets. The specific fluorescence of the bacteria by the fluorescent antibody method was observed in the cytoplasm of the epidermis, fat body and trachea of the infected larvae. The bacterial cells were found to multiply in the cytoplasm of those cells by electron microscopic observation.

**Arakawa A (1988). Western-blotting method for the detection of nuclear polyhedrosis virus in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 57:495-499.**

Two types of antisera agains *Bombyx mori* nuclear polyhedrosis virus (BmNPV) were prepared for the detection of the virus antigen by Western blotting method. One serum was raised to the purified BmNPV particles from alkaline dissolved polyhedra and the other type to the diseased larval hemolymph. The most preferable IgG concentration for this method was a 400 to 800 fold dilution. Virus proteins or polyhedral peptide were specifically detected by this method in the tests for purified virus and crude extract from the virus infected larvae and this method was useful to detect virus antigens from the silkworm larvae at 48 h after the virus inoculation.

**Iwashita Y and Zhou CQ (1988). Inactivation by the treatment of a nuclear-polyhedrosis virus of the silkworm, *Bombyx mori* with calcium hydroxide solution. *J. Sericult. Sci. Jpn.* 57:511-518.**

Procure alteration in the ultrastructure and the activity by the treatment of a nuclear polyhedrosis virus (NPV) with calcium hyudroxide solution was examined. Some nuclear polyhedra were dipped in saturated solution of calcium hydroxide and its serial diluted solution for various length of time. As a results, polyhedra dipped in saturated solution of calcium hydroxide were dissolved quickly and virions in the dissolving polyhedra were inactivated. When the silkworm larvae were inoculated with this soltion through peroral administration, no NPV infected larvae were observed. The process of polyhedra dissolution and of inactivation of the embedded virions in the calcium hydroxide solution was observed by the electron microscope. At first, polyhedron formed channel dissolution from the outer layer to the inner part, and the solution penetrated through the channels. The polyhedron protein in regions around the embedded virions degenerated gradually. During the process, the nucleocapsids of embedded virions were dissolved and the membranes of virions disintegrated completely.

## Vol. 58 1989

**Ichida M (1989). Relationship between application of a juvenoid and the incidence of nuclear polyhedrosis. *J. Sericult. Sci. Jpn.* 58:163-164. [Japanese]**

**Arakawa A (1989). Diagnosis of nuclear polyhedrosis by latex agglutination test in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 58:257-258. [Japanese]**

**Ichida M and Izaki T (1989). Susceptibility of the silkworm strain "Akebonon" to the *Bombyx mori* viruses. *J. Sericult. Sci. Jpn.* 58:259-260. [Japanese]**

**Kawase S (1989). Densonucleosis viruses of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 58:295-301. [Japanese-Review]**

**Kawase S (1989). Recent research on the infectious flacherie virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 58:363-373. [Japanese-Review]**

**Iizuka T and Terae N (1989). Peptide map analysis of insecticidal crystal proteins produced by strains of *Bacillus thuringiensis* by SDS-PAGE. *J. Sericult. Sci. Jpn.* 58:308-314.**

Insecticidal crystal proteins produced by the typical strains of *Bacillus thuringiensis* (subsp. *thuringiensis* Berliner, subsp. *kurstaki* HD-1, SUBSP. *kurstaki* HD-73, subsp. *sotto*, subsp. *dendrolimus*, subsp. *aizawai* IPL, subsp. *darmstadiensis*, subsp. *israelensis* and subsp. *kyushuensis*) were analyzed by SDS-PAGE and V8 protease-PAGE. Molecular weights of protoxins resulted from SDS-dissolution of the parasporal inclusions of SDS-dissolution followed by V8 protease digestion, were compared. The peptide maps of the protoxins demonstrated by the present study, could be one of the most reliable and useful characters for the identification of *B. thuringiensis* strains.

**Kobayashi M, Yanagawa S and Tanaka K (1989). Synthetic study on nucleopolyhedrosis control of the field in *Antheraea yamamai*. *J. Sericult. Sci. Jpn.* 58:344-348.**

It has been found that repeated outdoor rearing of wild sw at a single farm causes contamination of the farm with NPV, which results in unstable production. Therefore, a synthetic evaluation was performed on the cause of the disease and methods for providing stable production, such as the possibility of transmitting the viruses by insects inhabiting the farm and a method to inactivate the pathogen in soil and rearing trees. The results obtained are as follows. 1) Ants and aphides, high-density inhabitants of the farm, aggravated the transmission of the disease's viruses. 2) These viruses were found remaining on the branches and leaves of the red oak tress after rearing was completed. 3) Most of remaining viruses in soil were found to be accumulated in the surface layer to a depth of about 2 cm. 4) The remaining viruses in soild could be inactivated using a flame throwing sterilization device (tip temp 1,800°C). 5) The decrease in sw production during the young stages was mostly due to destruction by ants.

**Mike A, Ohmura H, Ohwaki M and Fukada T (1989). A practical technique of pebrine inspection by microsporidian spore specific monoclonal antibody sensitized latex. *J. Sericult. Sci. Jpn.* 58:392-395.**

The latex agglutination test was employed to detect the Microsporidian spores in *Nosema bombycis* (Nb), *Nosema* sp. M11 (M11) and *Vairimorpha* sp. M12 (M12) infected mother moth. On the slide agglutination test, Nb, M11 and M12 spore specific monoclonal antibody sensitized latex particles were specifically attached to the spores isolated from homogenate of Nb, M11 and M12 infected mother moth respectively. Our slide agglutination test using the monoclonal antibody sensitized latex particles will be useful for a practical technique of pebrine inspection.

**Watanabe H, Wang YX and Nagata M (1989). Comparative susceptibilities to a nuclear-polyhedrosis virus in the silkworm, *Bombyx mori*, reared on mulberry leaves and artificial diets. *J. Sericult. Sci. Jpn.* 58:407-411.**

Variations in susceptibilities of the various silkworm strains to peroral infection with a nuclear polyhedrosis virus (NPV) were relatively large when the larvae were fed on artificial diets instead of mulberry leaves. The larvae reared on artificial diets were more susceptible to peroral infection with NPV than those reared on mulberry leaves and the difference in susceptibility between them increased with larval age. However, no difference in susceptibility to intra hemocoelic infection with NPV was observed of the both larvae, suggesting food quality is important in the susceptibility of larvae to peroral infection with NPV. The gut juice from the larvae fed on mulberry leaves was significantly higher in the antiviral activity than that from the

larvae fed on artificial diets. The antiviral activity of the gut juice seemed to be depended on a protease.

**Abe Y and Iwashita Y (1989). Ultrastructures of *Leptomonas* sp. LA-SES-AA7601 strain pathogenic to the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 58:443-447.**

The amastigotes and the promastigotes of *Leptomonas* sp. LA-SES-AA7601 strain had numerous fine hairs on the cell surface. High electron density substances accumulated on the surface of the plasma membrane of the promastigotes in encystations. These features were considered to be different from *Leptomanas* spp. Which were previously reported.

**Iizuka T, Arakida M, Kikuta H, Isida K, Yueda I and Shikata E (1989). Electron microscopic observation of the plasmid DNA bearing insecticidal crystal protein gene in *Bacillus thuringiensis*. *J. Sericult. Sci. Jpn.* 58:448-456. [English]**

The native plasmids bearing the crystal protein (CP) gene of *Bacillus thuringiensis* were observed by electron microscopy. The size of several large plasmids from subspecies *sotto, kurstaki* HD-1 and *aizawai* IPL were determined by computer analysis. Several small size schimeric plasmids in which the CP gene (cryl-1) was closed were also observed and their size was compared with the reported size. It is shown that our estimation of the plasmid size was quite accurate. These electron microscopic observations of native plasmids of *Bacillus thuringiensis* and chimeric plasmids which ever are encoded for the CP gene, might be useful for identification of cloned plasmid.

**Utsumi S and Okada T (1989). Purification and amino acid composition of anti-*Enterococcus* protein (ASP) in the digestive juice of the silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 58:468-473.**

Anti-*Enterococcus* protein (ASP) contained in digestive juice (DJ) of silkworm larvae was purified to homogeneity by the treatments of ammonium sulfate precipitation, Sephadex G-50, DEAE-Sephacel, Sephadex G-50 column chromatography, and high performance of liquid chromatography (HPLC), DJ was collected from the starved 5$^{th}$ instar larvae, Asahi x Tokai, reared on mulberry leaves. About 4.9 mg of ASP was obtained from 10 ml of DJ with recovery of 36.7%. Molecular weight of purified ASP which indicated a single peak by HPLC treatment was estimated as 10,800 Da by SDS-PAGE analysis. Amino acid analysis indicated the predominance of hydrophobic and acidic residues, and the ratio of amino acid composition was similar to that of Sarcotoxin than Cecropin or Lepidopteran among the defense antibacterial proteins inducible in insect haemolymph.

## Vol. 59 1990

**Kawase S (1990). Recent Research on the cytoplasmic polyhedrosis virus of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 59:1-13. [Japanese-Review]**

**Yaginuma T, Kobayashi M and Kawase S (1990). Change in activities of several enzymes responsible for carbohydrate metabolism in midgut epithelium of the silkworm, *Bombyx mori*, infected with cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn.* 59:64-70.**

Activities of several enzymes responsible for carbohydrate metabolism were investigated in midgut epithelium of the silkworm, *Bombyx mori*, infected with cytoplasmic polyhedrosis virus (CPV). In CPV infected midgut, trehalase activity tended to increase during middle stage of the infection. Sucrose activity markedly decreased during late stage of the infection. Changing pattern in activity of L-leucyl-β-naphthylamidase was very similar to that in sucrase activity in CPV infected midgut. In contrast, glycogen phosphorylase activity increased during late stage of

the infection. Changing pattern in phosphorylase activity coincided with that in glycogen content in CPV infected midgut. These results suggest that in midgut cells, energy sources needed for the multiplication of CPV are provided from trehalose in haemolymph through a mediation of trehalase during middle stage of the infection and from glycogen in the cells through phosphorylase during the late stage.

**Ichida M (1990). Densonucleosis occurred in Kumamoto prefecture. *J. Sericult. Sci. Jpn.* 59:78-80. [Japanese]**

**Utsumi S, Okada T and Yano H (1990). ELISA of anti *Enterococcus* (Streptococcus) protein (ASP) in digestive juice of silkworm larvae, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 59:87-91.**

Anti *Enterococcus* protein (ASP) existed in the gut juice of the silkworm (*Bombyx mori*) larvae, was studied quantitatively by an enzyme linked immunosorbent assay (ELISA). The gut juice samples were prepared individually from the frozen larvae by dissection. There was relatively large variation in the concentrations of ASP among the larvae of the same strain. The ASP level in the silkworm strain KF20 and KF21 was compared. Apparently, the ASP concentration in the gut juice of KF21 was significantly higher than that of KF20. ASP in the larval gut juice was detected as low as 2.5 ng/ml.

**Kawarabata T, Hayasaka S, Ichida M, Segawa H, Teramine T, Uematsu H and Yasuda K (1990). Detection of microsporidians in adults if the cabbageworm *Pieris rapae crucivora* collected from mulberry fields. *J. Sericult. Sci. Jpn.* 59:288-292. [Japanese]**

**Asano S and Iizuka T (1990). Protoxin from diamond shaped crystals from *Bacillus thuringiensis* and insecticidal activity towards the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 59:375-380. [Japanese]**

**Kawase S (1990). Recent research on the nuclear polyhedrosis virus of the silkworm, *Bombxy mori*. *J. Sericult. Sci. Jpn.* 59:387-401. [Japanese]**

**Lin QL and Iwashita Y (1990). Replication of a nuclear polyhedrosis virus in the BM-N cell line of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 59:452-460.**

In order to analyse the mode of replication of nuclear polyhedrosis virus of the silkworm, *Bombyx mori*, (BmNPV), the BM-N cells which were derived from the silkworm ovary and infected with BmNPV were observed by light and electron microscopy.

The viruses were attached to the surface of the host cells and penetrated into the cells by endocytosis. The size of the nucleoli increased at 5 h postinoculation (pi). At 10 h pi, a PAP positive reaction showing the early stage of infection was observed. At 15 h pi, the nuclei became hypertrophied and Feulgen positive or methylgreeen stainable virogenic stroma was formed in the nuclei. From this virogenic stroma which developed and became electron dense a large number of nucleocapsids was produced. By 20 h pi, these nucleocapsids were released into the ring zone, enveloped with developmental membranes and grew to mature viruses. The nucleocapsids were also enveloped through budding from the nuclear membranes of the cell plasma membranes. At 72 h pi, the concentration of the viruses released into the culture medium became maximum. On the other hand, synthesis of the polyhedral protein was detected at 18 h pi and polyhedra at the early stage were formed in the ring zone at 30 h pi.

**Baba F, Asano S and Iizuka T (1990). Purification of crystals from *Bacillus thuringiencis* by using Percoll. *J. Sericult. Sci. Jpn.* 59:487-489. [Japanese]**

**Vol. 60 1991**

**Arakawa A (1991). Quantitative assay of cytoplasmic polyhedrosis virus in the feces of the silkworm, *Bombyx mori*, using enzyme linked immunosorbent assay (ELISA). *J. Sericult. Sci. Jpn.* 60:105-111.**

Enzyme linked immunosorbent assay (ELISA) was developed for the quantitative assay of *Bombyx mori* cytoplasmic polyhedrosis virus (CPV) in larval feces. The double antibody sandwich method was more sensitive than the indirect method and nonspecific reactions against the feces extract were not observed. Alkaline phosphatase was suitable for use as conjugate enzyme to the anti-CPV IgG, while peroxidase gave rise to non-specific reactions. The addition of 2% of polyvinyl pyrrolidone (MW 40,000) to the extraction buffer (100mM Tris-HCl, ph 8.0) was necessary to eliminate the background association with the coupling of feces extract to the microtest plate. The double antibody sandwich method enabled to detect 8 ng/ml of purified CPV using alkaline phosphatase conjugated ANTI-CPVIgG. The addition of 1% extract of non injected larval feces did not inhibit the reactions for the detection of CPV. There was a positive correlation between the amount of CPV in the feces and the value obtained by ELISA.

**Ishihara R and Iwano H (1991). The lawn grass cutworm, *Spodoptera depravata* Butler, as a natural reservoir of *Nosema bombycis* Naegeli. *J. Sericult. Sci. Jpn.* 60:236-237. [Japanese]**

**Iwano H and Ishihara R (1991). Dimorphic development of *Nosema bombycis* spores in gut epithelium of larvae of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 60:249-256.**

*Nosema bombycis*, when inoculated orally to the 2$^{nd}$ instar larvae of the silkworm, *Bombyx mori*, developed two types of spores (short polar tube spore=ST and long polar tube type spore=LT), identical with the spores revealed in cultured cells. One type, corresponded to the FC (spores with few coils) and the other type to the MC (spores with many coils) in the cultured cells, respectively. The significance of the dimorphism of *N. bombycis* was discussed and an outline of the development of *N. bombycis* was also presented.

**Iwano H and Ishihara R (1991). Isolation of *Nosema bombycis* from moths of the lawn grass cutworm, *Spodoptera depravata* Butler. *J. Sericult. Sci. Jpn.* 60:279-287.**

A strain of *Nosema bombycis* was isolated from the lawn grass cutworm, *Spodoptera depravata* Butler. Identificationa was based on the following characteristics, in culture, the cells were binucleate with a diplokaryotic arrangement throughout the development; spore formation was apansporoblastic, disporous and dimorphic. In one type of spores, the coil of the polar tube was characterised by 3.8 turns, while in the other by 11.8 turns. The former spores hatched spontaneously. The latter spore were oval in shape and 3.60 x 2.11µm in size. Immunologically the strain shared spore surface specific antigen(s) with *N. bombycis*. Infection within the larvae of *Bombyx mori* was systemic and the strain was transovarially transmitted to the next progeny. Less virulent than the reference strain of *Nosema bombycis*.

**Noguchi Y (1991). Electron Microscopic Investigations on the Multiplication of the Variants of *Bombyx mori* Nuclear-Polyhedrosis Virus. *J. Sericult. Sci. Jpn.* 60:302-309.**

The multiplication of three variants of *Bombyx mori* nuclear polyhedrosis virus (BmNPV), which were isolated from BmNPV - infected cells treated with a mutagen, 5-bromo-2'-deoxyuridine, was studied by transmission electron microscopy. No.15 and 16 variants exhibited a defect in the *de novo* synthesis of the envelope, and they formed few polyhedra devoid of virions. Since the nucleocapsids derived from the nuclei formed buds through the plasma membrane into the reticular structure with extended remarkably in the cytoplasm, they protruded

from the cytoplasm with the reticular structure. No.24 variant in which no polyhedron formation was observed, produced progeny viruses normally.

**Eguchi R, Ninaki O and Hara W (1991). Genetical analysis on the nonsusceptibility of densonucleosis virus in the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 60:384-389.**

In the silkworm, *Bombyx mori*, it was reported that some strains were not infected with densonucleosis virus type-1 (DNV-1) and this resistance appeared to be controlled genetically. Genetic analysis showed that the resistance to the densonucleosis virus type 1 was controlled by a recessive gene. The gene was designated as "non-susceptibility to densonucleosis virus type-1" gene with the symbol *nsd-1*. The *nsd-1* gene was located at the position 8.3 on the twenty first chromosome based on the three point analysis and the sequence of the genes was *rb, nsd-1, Sph*. The homozygote of *nsd-1* was completely resistant to the DNV-1.

**Yasunaga C, Funakoshi M, Kawarabata T and Hayasaka S (1991). Infection and development of *Nosema* sp. NIS M11 (Microsprodia: Protozoa) in a lepidopteran cell line. *J. Sericult. Sci. Jpn.* 60:450-456.**

Spores of *Nosema* sp. NIS M11 primed with a 0.1 N KOH solution were inoculated to *Antheraea eucalypti* cell cultures. The percentage of germinated spores was less than 30% and the initial level of infection in the inoculated cells was about 10% in this study. Growth and development of the sporoplasms emerging from *N.* sp. NIS M11 spores appeared to be normal in the *A. eucalypti* cells. The life cycle of *N.* sp. NIS M11 observed *in vitro* was similar to that of *N. bombycis*. However, the spread of infection and spore production in the *A. eucalypti* cell cultures infected with *N.* sp. NIS M11 were markedly different from those of *N. bombycis*.

**Asano S and Iizuka T (1991). Identification of insecticidal activity of *Bacillus thuringiensis* against the silkworm by DNA hybridization method. *J. Sericult. Sci. Jpn.* 60:475-479.**

In order to identify the insecticidal activity of 20 *Bacillus thuringiensis* subspecies which produce bipyramidal crystals, dot blot hybridization of DNA purified from the subspecies was performed with a cloned crystal protein gene (cry1-gene) fro subsp. *kurstaki*HD-1. The DNAs from the ten subspecies exhibiting on insecticidal activity against the silkworm *Bombyx mori*, strongly hybridized with the probe prepared from the cloned crystal protein gene. However, DNAs from the other ten subspecies lacking insecticidal activity did not hybridize with the probe. Similar results were also obtained in the experiment using a probe prepared from the DNA fragment that is specific for insecticidal activity. Based on these results, we concluded that the intensity of the insecticidal against the silkworm can be determined DNA hybridization.

## Vol. 61 1992

**Lin YQ and Iwashita Y (1992). Histopathological Investigation on the replication of Two Nuclear Polyhedrosis Viruses of *Antheraea yamamai* and *Spodoptera litura* in Larvae of *Antheraea pernyi* and *Spodoptera litura*. *J. Sericult. Sci. Jpn.* 61:295-299.**

*In vivo* pathway of the infection and replication of two nuclear polyhedrosis viruses of *A. yamamai* and *S. litura* in the midgut and epidermal cells was studied by EM. The replication pattern of the virus in the epidermal cells differed fm that in the midgut cells. Nucleocapsids produced in the nuclei of the epidermal cells were enveloped and occluded in polyhedra. However, few nucleocapsids produced in the nuclei of the midgut cells acquired an envelope and become occluded in polyhedra. Instead a large no of nucleocapsids budded through the nuclear membrane or nuclear pores and entered the cytoplasm. The nucleocapsids moved to the basal plasma membranes and budded into the hemocoel via basal lamina. These budded viruses become attached and penetrated into host cells in the hemocoel and started new replication.

**Kawakami Y, Inoue T, Kikuchi M, Takayanagi M, Sunairi M, Ando T and Ishihara R (1992). Primary and secondary structures of 5S Ribosomal RNA of *Nosema bombycis* (Nosematidae, Microsporidia). *J. Sericult. Sci. Jpn.* 61:321-327. [English]**

*Nosema bombycis*, a notorious parasite of the silkworm (*Bombyx mori*), which is the type species of the genus Nosema and belongs to Microsporidia, is unique among eukaryotes in having prokaryotic features, particularly of its ribosomes. To analyise its phylogenic and taxonomic position, 5S rRNA and its gene were sequenced by limited digestion with RNase and by the usual DNA sequencing of the cloned 5S rRNA gene which was amplified by PCR (polymerase chain reaction), respectively. The results indicated that the 5S rRNA of *N. bombycis* displays a typical eukaryotic structure.

**Shimizu S, Tsuchitani Y and Matsumoto T (1992). Purification and properties of an extracellular protease from *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 61:421-428. [English]**

*Beauvaria bassiana* F18 grown in a medium with *Bombyx mori* exuviae as the sole carbon and nitrogen source produced an extracellular protease. The extracellular protease was purified about 19 fold, with a recovery of 20.5% by a procedure that included column chromatography on CM-Toyopearl and Sephacryl S-100 gel filtration. The purified protease gave a single protein band with a molecular weight of 32,000 Da on SDS-polyacrylamide gel electrophoresis. It was optimally active at pH 9.5 and in the temperature range of 37 to 42°C. The enzyme activity was almost completely lost at 60°c within 15 min. Its enzymatic activity was that of an endopeptidase which hydrolysed elastin but was much less active on casein, bovine serum albumin, fibrinogen and γ-globulin. It was inhibited by phenyl methlysulfonyl fluoride, suggesting that the enzyme contains a serine residue in the active center of the protein molecule. The protease activity was not affected by metal chelating agents, trypsin inhibitor, and chymotrypsin inhibitor.

## Vol. 62 1993

**Shimane T and Kawakami K (1993). Safety tests for the use of an entomogenous fungus *Beauveria brongniartii*. *J. Sericult. Sci. Jpn.* 62:30-37.**

In order to utilize as a microbial insecticide, the entomopathogenic fungus, *Beauveria brongniartii* (*B. tenella*) isolated from dead adults of the yellow-spotted longicorn beetle, the pathogenicity to and other harmful effects of this fungus on the silkworm and mouse were investigated in this tests. When 21 isolates of this fungus were inoculated individually to 4$^{th}$ instar larvae of the silkworm by dipping them into a conidial suspension ($10^7$~$10^8$ conidia/ml), all the treated larvae survived and pupated subsequently. The fungus was not pathogenic to the larvae, while *B. bassiana* showed a marked pathogenicity. In safety tests for the mouse, the animals were subjected to intraperitoneal injections as well as oral and respiratory administration of a large amount of conidia of this fungus. All the mice survived and exhibited a normal appearance and behaviour. Weight gain of the treated mice was almost similar to that of the controls. There was no indication of pathogenicity to and other harmful effects on the mouse. Conidia of the fungus did not germinate at 35°C, and they died within seven days under the thermal condition.

**Abe H, Yokoyama T, Oshiki T and Kobayashi M (1993). Analysis of the mechanism of nonsusceptibility to the densonucleosis virus (densovirus) type-2 in the silkworm, *Bombyx mori*, using a genetic mosaic. *J. Sericult. Sci. Jpn.* 62:38-44.**

BmDNV-2 (type-2) multiplies in the nuclei of the columnar cells of the midgut epithelium of the sw. Some sw strains are genetically nonsusceptible (completely resistant) to BmDNV-2 infection even after the administration of a high dose. This non susceptibility is controlled by a recessive gene (*nsd-2*: nonsusceptibility to BmDNV-2). In order to investigate whether the

nonsusceptibility is controlled by the *nsd-2* gene is determined at the whole body level or cellular level, susceptible/non-susceptible bilateral mosaic larvae were produced using the hereditary mosaic strain (*mo*) and we administered BmDNV-2. By immunoperoxidase staining of the larval body section, five mosaic larvae displaying midgut mosaicism were detected in two areas, infected (*nsd-2*/+) and noninfected (*nsd-2*/*nsd-2*) respectively. These results indicate that the nonsusceptibility is determined by a single gene at the single cellular level.

**Shimizu S, Higashiyama R and Matsumoto T (1993). Chromosome length polymorphisms in *Beauveria bassiana*. *J. Sericult. Sci. Jpn.* 62:45-49. [English]**

Experimental conditions for the separation of chromosomal DNAs from *Beauveria bassiana* by pulsed-field gel electrophoresis (PFGE) were established. The chromosomal DNA molecules of *Beauveria bassiana* have been separated into six bands by PFGE. Using the *Schizosaccharomyces pombe* chromosomesas size standard, we estimated the size of these chromosomal DNAs to be from 2.4 to 7.9 magabase pairs (Mbp) and total genome sizes of the three isolates from 26.1 to 30.4 Mbp. Significant differences in the size of each chromosome existed among the isolates of *B. bassiana*.

**Shimane T and Kawakami K (1993). Susceptibility of adults of yellow spotted longicorn beetle, *Psacothea hilaris* PASCOE (Coleoptera: Cerambicidae), reared with an artificial diet through their larval stage, to an entomogenous fungus *Beauveria brongniartii*. *J. Sericult. Sci. Jpn.* 62:68-70. [Japanese]**

**Shimane T and Kawakami K (1993). Virulence of *Beauveria brongniartii* conidia and hyphal bodies against the yellow-spotted longicorn beetle, *Psacothea hilaris*. *J. Sericult. Sci. Jpn.* 62:71-74. [Japanese]**

**Asano S, Bando H, Kikuta H and Iizuka T (1993). Identification of cry II genes in *Bacillus thuringiensis* isolates by the use of ligo-nucleotide DNA primers. *J. Sericult. Sci. Jpn.* 62:210-215.**

Experiments were conducted on 8 *Bacillus thuringiensis* (Bt) isolates identified by KIKUTA (1990). Observation by SEM showed the presence of a large number of cuboidal crystals compared to the bipyramidal ones. Identification by serotyping was performed and the structure of the cryI and cryII genes in these isolates was determined by the application of the method of oligonucleotide primer synthesis and PCR amplification.

Characteristic profiles of Acp10-4 and GSK1-1 belonging to *Bacillus thuringiensis* subsp. *kenyae* revealed only the presence of the cryII A gene. The other 6 strains belonging to the subsp. *kursaki* and *galleriae* harboured the cryII A and cryII B genes. The mosquitocidal activity against *Aedis japonicus* of Acp10-4 and GSK1-1 was much higher than that of the other strains.

The amount of 135 and 65 kDa protoxins consisting of bipyramidal and cuboidal crystals, respectively was determined by SDS-PAGE with a densitometer. The effect of a larger amount of 135 kDa protoxins than of 65 kDa protoxins did not affect the expression in these strains.

**Asano S, Bando H and Iizuka T (1993). Amplification and identification of cry II genes from *Bacillus thuringiensis* isolates by PCR procedure. *J. Sericult. Sci. Jpn.* 62:223-227.**

Oligonucleotide primers including a specific domain of *cry II* gene sequences were synthesized in order to identify *cry II* genes which encode the expression of the P2 protein in *Bacillus thuringiensis* (Bt) strains. The *cry II* genes of the Bt isolates were amplified by polymerase chain reaction (PCR) and the amplified DNA was used as DNA probe for identification. *Cry II B* was distinguished from *cry II A* by *Hinc II* digestion. The *cey II* genes in

the Bt isoltes were identified by agarose gel electrophoresis. Based on the identification of the Bt subspecies, *kurstaki* HD-1, *aizawai* IPL and *galleriae* harboured both *cry II A* and *cry II B* genes, while *kenyae* harboured only the *cry II A* gene. This procedure was effective for the identification of Bt isolates including both lepidopteracidal and dipteracidal proteins.

**Arakawa A (1993). Monitoring of the appearance of the cytoplasmic polyhedrosis virus in the feces of the silkworm larvae associated with viral contamination of the rearing bed. *J. Sericult. Sci. Jpn.* 62:319-324.**

The spread of CPV in the sw rearing bed was investigated by the detection of the CPV in the feces excreted by the larvae. The incidence roused from 23.9% or 10.4%~11.9% 12 days after the inoculation of 2% or 0.2% of the virus to healthy larvae respectively. The higher the conc of virus in the feces, the higher the ratio of larvae with the disease. Two to 3 days after the rearing of the larvae inoculated with the virus, more than 10μg of virus was detected by ELISA in 1 g of feces in the rearing bed. A high degree of contamination was detected in the center of the rearing bed, where the virus mixture had been prepared for larval inoculation until the 6$^{th}$ day. Thereafter the space of contamination was higher in the periphery of the rearing bed.

**Abe H, Kobayashi K, Shimada T, Yokoyama T, Maeda S, Hamano K, Oshiki T and Kobayashi M (1993). Infection of a susceptible/nonsusceptible mosaic silkworm, *Bombyx mori*, with densonucleosis virus type-2 is not lethal. *J. Sericult. Sci. Jpn.* 62:367-375.**

BmDNV-2 (type-2) multiplies in the nuclei of the midgut epithelium columnar cells of the sw. The susceptibility is controlled by the recessive gene, *nsd-2*. Certain sw strains are genetically non-suceptible to DNV-2. Mosaic sw larvae with susceptible (*nsd-2*/+) and nonsusceptible (*nsd-2*/*nsd-2*) midgut cells were produced using the hereditary mosaic strain (*mo*). 17 newly ecdysed 2$^{nd}$ instar mosaic larvae were fed with DNV-2 and the infection was monitored using PCR to detect DNV-2 in the feces. DNV-2 multiplied continually in five of the larve, however, they survived and grew showing no outer symptoms of virus infection. Immunoperoxidase and Feulgen light-green staining of larval body sections indicated that DNV-2 infection only occurred in the regions with suceptible cells (*nsd-2*/+). These results indicate that DNV-2 infection itself is not lethal.

**Abe H, Shimada T, Kobayashi K, Maeda S, Yokoyama T, Oshiki T and Kobayashi M (1993). Detection of densonucleosis virus in the silkworm, *Bombyx mori*, from fecal specimens by a polymerase chain reaction. *J. Sericult. Sci. Jpn.* 62:376-381.**

Feces from sw larvae infected with BmDNV-1 (type-1) and BmDNV-2 (type-2) contain viruses particles. A PCR reaction was used to detect DNV infected sw larvae without killing. Newly ecdysed 4$^{th}$ instar larvae of susceptible and nonsusceptible strains were fed DNVs 24 h prior to the collection of feces. Feces were collected daily and DNA extracts from a single specimen of fecal matter was used as a template for PCR. In the susceptible strains, DNVs were detected in all fecal speciments beginning the day after inoculation to the day of viral death. In the non-susceptible strains, DNVs were detected only in fecal matter collected the day after inoculation and were not detected on and after 2$^{nd}$ day. Based on these studies, we were able to diagnose rapidly both DNV-1 and DNV-2 infection using PCR to detect DNVs in fecal matter.

**Nakagaki M, Tsuda H, Kajiura Z and Takei R (1993). Localization of DNV infection in the midgut of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 62:405-411.**

Time course of the accumulation of viral structural polypeptides was investigated in the larval midgut of the sw infected with two groups of DNVs. BmDNV-1 (Ina strain) and BmDNV-2 (Yamanashi strain). Viral structural polypeptide with a molecular weight of approx. 57,000 (57 K polypeptide) was detected in the epithelial cells of the DNV-1 infected midgut and a viral

polypeptide with a mol wt of approx 53,000 (53 K polypeptide) was detected in the epithelial cells of DNV-2 infected midgut by immunoblot analysis using anti-DNV-1 or anti-DNV-2 serum. Kinetics of the accumulation of the 57 K and 53 K polypeptides in the infected midgut epithelium was similar. Viral polypeptides could be detected 2 days after pi in the posterior part, 3 days pi in the mid part and 6 days pi in the anterior part of the midgut. These results indicate that DNV infection occurred first in the posterior part of the midgut and spread to the mid and anterior parts of the midgut.

**Suzuki S and Satoh T (1993). On the easy detective method for *Aspergillus* fungi in silkworm rearing house by means of using filter paper containing medium and poly-ethylene bag. *J. Sericult. Sci. Jpn.* 62:517-522. [Japanese]**

**Kobayashi J and Belloncid S (1993). Efficient lipofection method for transfection of the silkworm cell line, NISES-BoMo-15AIIc, with the DNA genome of the *Bombyx mori* nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 62:523-526. [English]**

The silkworm, *Bombyx mori*, cell line (BoMo-15AIIc) (Inoue *et al*, 1990), susceptible to the BmNPV and several cytoplasmic polyhedrosis viruses (CPVs) is commonly used for various research purposes such as for the baculovirus mediated expression of recombinant proteins (KOBAYASHI *et al*, 1990, 1992) for studies on the interaction between CPVs and BmNPV during their replication *in vitro* and in experiments leading to the development of CPV expression vector. All these studies require the use of highly efficient methods for the transfection of viral genomes into the cells.

Using several mammalian cells, FELGNER *et al*, (1987) demonstrated that the DOTMA {n-[1-(2, 3-dioleyloxy) propyl]-N, N, N-trimethyl ammonium chloride} mediated transfection (lipofection) method was more efficient for both stable and transient expression of the introduced DNA than either the DEAE-dextron or calcium phosphate-mediated transfection. The increase of the concentrations of DOTMA containing lipid that improved transfection, however, was toxic and this toxicity varied with the type of cells, the duration of exposure to DOTMA, and the density of cell culture. Therefore, the optimization of the technical conditions of lipofection for each cell line is recommended in order to achieve a high efficacy of transfection. Lipofection method has already been applied successfully for the transfection of *Spodoptera frugiperda* (sf-9) insect cells with the DNA genome of *Autographa californica NPV (AcNPV)* (Patel *et al*, 1992). In order to improve the transfection efficiency and broaden the variety of manipulation techniques for the BoMo-15IIc cell line, we applied the lipofection method to this cell line.

## Vol. 63 1994

**Lu X, Hashimoto Y, Shimizu S, Matsumoto T and Maekawa S (1994). Serotyping of *Enterococcus faecalis* isolated from larval intestine of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 63:330-332. [Japanese]**

**Shimada T, Chan IC, Noguchi Y, Nagata M and Kobayashi M (1994). Structural and functional abnormality of the polyhedrin gene in a polyhedron-deficient mutant of the *Bombyx mori* nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 63:353-360.**

We compared the structure and function of the polyhedrin gene of *Bombyx mori* nuclear polyhedrosis virus between a polyhedron defective mutant and the wild type. Western blotting analysis using an anti-polyhedron antibody demonstrated that the polyhedron defective mutant did not synthesize polyhedrin in the fat body cells of the infected silkworm. Northern blotting analyses using a polyhedrin gene fragment as a probe showed that the fat body cells infected with the mutant virus did not synthesize mRNA coding for polyhedrin at all, while the cells infected with the wild type virus synthesized a large amount of polyhedrin mRNA on days 3 and 4 after

infection. In Southern blotting experiments, however, DNAs from both mutant and wild type viruses hybridized with the polyhedrin gene probe. DNA sequencing revealed that the polyhedrin gene and its upstream region in the mutant and wild type viruses hybridized with the polyhedrin gene probe. DNA sequencing revealed that the polyhedrin gene and its upstream region in the mutant had 5 point mutations. A conserved motif ATAAG of the very late gene promoter of the baculovirus was changed to ATAAA in the mutant. This alteration may be responsible for the lack of polyhedron formation.

**Sasaki J, Asano S, Bando H and Iizuka T (1994). Characteristics of *Bacillus thuringiensis* isolated from soil in Hokkaido area of Japan. *J. Sericult. Sci. Jpn.* 63:361-366.**

A total of 63 isolates of *Bacillus thuringiensis* were isolated from 187 soil samples collected in Hokkaido area of Japan. Of these isolates, 45 were identified as seovars *alesti, kurstaki, sotto, aizawai, israelensis, Indiana* and *mexicanensis* by H antisera and 18 were untypable because of nonmatility. The aim of this study was to isolate novel strains with different spectra of activity or high toxicity. Isolates were characterised by the morphology of parasporal crystals, bioassay against *Bombyx mori* and identification of *cry* genes by PCR. Using these techniques, 3 novel strains were obtained. The isolate serovar *mexicanensis* TKD2-14, produced cubodal crystals and the isolate serovar *alesti* KMK9-20 with bipyramidal crystals attaching to spores were different from the type strains. In addition, the isolate serovar *sotto* KMK1-36 produced bipyramidal crystals similar to the reference strain. However, the composition of the *cry* genes of this isolate was *cryIA(b)* and *cryIA(c).*

**Noguchi Y, Kobayashi M and Shimida T (1994). An application of the polymerase chain reaction for practical diagnosis of the nuclear polyhedrosis in large scale culture of *Bombyx mori*. *J. Sericult. Sci. Jpn.* 63:399-406.**

We developed a simple and highly sensitive method for the detection of the nuclear polyhedrosis virus in a large scale culture population of *Bombyx mori*, using the polymerase chain reaction (PCR). Newly exuviated 3$^{rd}$ instar larvae were crushed in plastic bags, mixed with 8 volumes of alkali solution (0.2 M $Na_2CO_3$, 0.2 M NaCl, 20 mM EDTA, ph 10.8) and incubated at 25°C for 30 min to dissolve the polyhedra. The lysate was treated with detergent and Proteinase K. DNA was purified with phenol, diluted with water and used as a template for PCR. We could detect a single polyhedrosis larva in a population of 20,000 larvae even if the sample contained mulberry leaves or rearing wastes. Because our method is much more sensitive than other conventional methods, we propose it as a tool for a practical diagnosis of the nuclear polyhedrosis in cooperative rearing.

**Lu X, Hashimoto Y, Matsumoto T and Maekawa S (1994). Taxonomical studies on *enterococci* isolated from the intestine of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 63:481-487.**

Upto 99 *Enterococcus* strains were isolated from the intestine of healthy silkworm larvae and moths. Except 7 strains which did not grow in a medium at pH 9.6, the remaining 92 strains were classified into 8 groups (I-VIII) on the basis of the differential characteristics of the group D enterococci and of the virulence to insects. Major strains were identified to be the *E. faecalis* type 1 which were non-hemolytic and had ability to produce a large amount of extracellular proteinase and to agglutinate with an anti-*E. faecalis* antiserum. These included yellow pigmented *E. casseliflavus* and *E. durans*, as well as some unidentified strains. Also hemolytic strains were isolated from healthy silkworms, providing the first evidence as to the occurrence of *β*-hemolysis strains in the insect oriented enterococci.

## Vol. 64 1995

**Inoue S, Yasunaga C, Funakoshi M, Kawarabata T and Hayasaka S (1995). Infection and development of *Vairimorpha* sp. NIS M12 (Microsporidia: Protozoa) in a lepidopteran cell line. *J. Sericult. Sci. Jpn.* 64:39-45.**

*Vairimorpha* sp. NIS M12, a microsporidian parasite isolated fm the sw, successfully infected the cell line of *Spodoptera frugiperda* SF21AE II. After priming with 0.1 N KOH solution, the spores were mixed thoroughly with *S. frugiperda* cells suspended in Rinaldini's solution. Inoculated cells wre cultured at 28°C in IPL-41 medium supplemented with 10% fetal bovine serum. At a ratio of 30 spores per cell, 9.1% of *S. frugiperda* cells were infected initially with sporoplasms 1 h pi. About 65% of the infected cells harboured a single sporoplasm. The spread of infection and spore production of *V.* sp. NIS M12 in cell culture were markedly different fm those of *N.b.*, while in the *in vitro* life cycles of the two parasites were similar. Short-coiled spores occurred 72 h pi and long coiled mature spores were produced 10 days pi. No formation of syncytia was observed in *Vairimoropha* infected cell cultures.

**Hashimoto Y, Yanase T and Matsumoto T (1995). Interference of BmNPV replication by the viruses altered by serial undiluted passage in cell culture. *J. Sericult. Sci. Jpn.* 64:150-155.**

A plaque-purified strain, D1, of BmNPV was subcultured in BmN4 cells by 34 series of passage without dilution. During the passage cycles between 10 and 17 (P10 and P17), the titer of infectious, standard nonoccluded virus (NOV) decreased to 6.0 x $10^6$ $TCID_{50}$/ml, which was 100-fold less than that of the original (P0) inoculation. Thereafter, the titer remained low with slight fluctuation until P34. The cytopathic effect in the BmN4 cells infected with the subcultured inocula changed drastically; at P18, cells with reduced number of polyhedra appeared and at P31, cells without polyhedra became dominant. To see if the subcultured viruses interfere the replication of infectious NOV, the P0 inoculum and the P34 inoculum were mixed in various ratios at a constant NOV titer and infected to BmN4 cells. The higher the P34/P0 ratio was, the lower the titer of the viruses in culture media became, suggesting that the defective interfering particles (DIPs) were formed during the serial passage of the P0 virus inoculum in cell culture.

**Kawakami Y, Inoue T, Uchida Y, Hatakeyama Y, Iwano H and Ishihara R (1995). Specific amplification of DNA from reference strains of *Nosema bombycis*. *J. Sericult. Sci. Jpn.* 64:165-171. [English]**

An attempt was made to develop a new system for pebrine inspection. DNAs from the reference strains of a microsporidium, *Nosema bombycis*, were specifically amplified by polymerase chain reaction (PCR) using primers from a putative pseudogene together with those derived from conserved regions of a small subunit rRNA gene of *Nosema bombycis*. Six microsporidia were tested: 3 *N.nosema bombycis* strains, ie, the reference strains SES-NU and NIS-001 and a strain named Sd-NU-IW8401 which was isolated from *Spodoptera depravata* and 3 species ie, *Nosema* sp. isolate NIS-M11, *Vairimorpha* sp. isolate NIS-M12 and *Pleistophora* sp. isolate Pl-NU. When PCR was conducted using DNA from these 6 microsporidia as templates, PCR products were obtained only from SES-NU and NIS-001 which were reference strains of *N. bombycis*.+

**Hayasaka S and Inoue H (1995). Polyhedra formation in the midgut tissues of the silkworm, *Bombyx mori*, infected with a nuclear polyhedrosis virus. *J. Sericult. Sci. Jpn.* 64:224-229.**

The *in vivo* multiplication of NPV in the midgut tissue of the silkworm, was observed with special reference to the polyhedra formation and the results were compared with *in vitro* events.

In the midgut tissue of the larvae which were treated at 5°C for 24 h before virus inoculation, the polyhedra formation was accelerated but the incorporation of matured viral rods into polyhedra was very few. The viruses markedly underwent budding from the infected midgut cells to the hemocoel. The virus envelopments were abnormal and large virus-envelops showed affinity to polyhedra; in some cases the virus-envelops wrapped the polyhedra at which nucelocapsids showed affinity.

**Noguchi Y (1995). Isolation and characterization of temperature sensitive mutants of *Bombyx mori* nuclear polyhedrosis virus and their multiplication in a cell line and larvae of *Bombyx mori*. *J. Sericult. Sci. Jpn.* 64:230-236.**

Temperature sensitive (ts) mutants (No.128 and No.217) of BmNPV were isolated by the plaque formation method from BmN4 cells after the inoculation of BmNPV which had been treated with 5-bromo-2'-deoxyuridine. These 2 ts mutants were characterized, together with a previously established one 9No.20). At 25°C the yield of the progeny viruses of 3 ts mutants did not differe from the wild type of BmNPV. At 33°C, the rates of viral replication of the mutants No.20 and No.128 were markedly inhibited. Multiplication of the mutant No.217 was inhibited slightly and the polyhedron formation and viral replication were decreased. Also observed was abnormal virus-envelope formation in the fat body cells. In the case where culture temp was shifted from 25 to 33°C, pi of the ts mutants, viral replication was inhibited both in the cell line and in larvae. When the temp was shifted back to 25°C, the ts mutants again replicated normally in the cell line and in larvae. These results indicated that the inhibition of virus replication was not caused by the defect in the viral absorption pathway. Coinfection of the ts mutant No.128 and others exhibited a complementation phenomenon and polyhedra formed were increased at 33°C.

**Yasui H and Shirata A (1995). Detection of antibacterial substances in insect gut. *J. Sericult. Sci. Jpn.* 64:246-253.**

Insects exposed to many microbes have defense mechanisms against them. While making inquiries into the defense mechanism, antimicrobial substances in insect guts (3 species: *Bombyx mori*, *Psacothea hilaris* and *Locusta migratoria*) were detected by the direct method and the silicagel thin layer chromatography (TCL) method. Within the subjected 44 microbes, *Bacillus subtilis*, *Bacillus cereus* and *Pyricularia oryzae* were very sensitive, and a bacterium isolated from the guts of silkworm larvae and 6 more microbes were sensitive against acetone extracts of the insect gut contents. Other pathogenic microbes, especially all of the insect pathogenic fungi, were resistant. *B. subtilis* was the best microbe species for detection of antimicrobial substances because it produced clear antimicrobial spots in both methods. Using the TCL method, antimicrobial spots in acetone extract of leaves were detected and additional spots were detected in the insect gut contents. Furthermore, one of the spots of mulberry leaves did not appear when the silkworm gut contents were tested. These results indicate that the metabolism of antimicrobial substances occurs in the insect gut, resulting in the changes of the TLC pattern. Acetone extracts of the inset gut contents showed strong antimicrobial activities using the direct method. The acetone extract of mulberry leaves had antimicrobial activities and that f the mixture of mulberry leaves and the bacteria isolated from the guts of longicorn adults which ingested mulberry leaves showed stronger antimicrobial activities. Since the insect digestive juice did not have such strong antimicrobial activities, antimicrobial substances in the leaves and those converted from leaf substances might contribute to the insects' resistance against some microbes.

**Abe H, Shimada T, Tsuji T, Yokoyama T, Oshiki T and Kobayashi M (1995). Identification of random amplified polymorphic DNA linked to the densonucleosis virus type-1 susceptibility gene of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 64:262-264. [Japanese]**

**Noguchi Y, Kobayashi M and Shimada T (1995). An improved method for PCR-based detection of nuclear polyhedrosis virus in *Bombyx mori*. *J. Sericult. Sci. Jpn.* 64:352-359.**

We have already established a diagnostic technique based of PCR to detect NPV in a sw population using samples containing the wastes and feces as well as larval bodies. In the present study, we utilized potassium acetate precipitation, instead of phenol/chloroform treatment at the deproteinisation step of DNA extraction, thus avoiding danger of applying a large amount of organic solvents. Also HCl treatment of DNA followed by ethanol precipitation was found to be effective to increase the diagnostic sensitivity. By using this improved method, we could detect a single polyhedrosis-infected larva in a 0.5, 1 and 3 kg sample for the $1^{st}$, $2^{nd}$ and $3^{rd}$ instar, respectively.

**Furuta Y (1995). Stability of silkworm viruses to long-term refrigeration. *J. Sericult. Sci. Jpn.* 64:395-398. [Japanese]**

**Choi HK, Taniai K, Kato Y, Okuda KK, Yamamoto M, Chowdhury S, Xu J, Sugiyama M, Choi SK, Miyanoshita A, Debnath NC, Asaoka A, Ishii T and Yamakawa M (1995). Induction of activity of protein kinase C and A by bacterial lipopolysaccharide in isolated hemocytes from the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 64:450-456. [English]**

An *in vitro* system containing *Bombyx mori* hemocytes, mainly granular cells and plasma cells, was used to examine the induction of protein kinase C (PKC) and protein kinase A (cAMP-dependent protein kinase A, PKA) activities by various kind of stimuli. The PKC activity was clearly detected after the hemocytes were treated with lipoplysaccharide (LPS) from *Escherichia coli*, inomycin, cholera toxin from *Vibrio cholerae* or 4 β-phorobor 12-myristate 13 acetate (PMA). Hoever, the activity was not detected in the absence of these stimuli. These results suggest that not only LPS but also $Ca^{2+}$ and a G protein may be involved in the signal transduction for the induction of PKC activity. Likewise, the PKA activity was observed in the hemocytes treated with LPS, dibutylryl cyclic adenosine monophosphate (dcAMP), inomycin or cholera toxin, whereas non-treated control cells did not show the activity. Thus LPS, cyclic AMP, $Ca^{2+}$ and a G protein were likely to participate in the signal transduction for the induction of PKA activity. Western blot analysis using monoclonal antibodies against rabbit brain PKC-α showed that hemocyte samples treated with LPS contained a ca. 90 kDa protein which cross-reacted with the antibodies, but this protein was not present in a LPS non-treated sample. PKC and PKA in *Bombyx mori* hemocytes may be activated *in vivo* by LPS upon bacterial infection, resulting in triggering the self-defense reactions such as antibacterial protein gene expression.

**Hara T, Tarui H, Kawaguchi H, Yoshimura N and Kawarabata T (1995). Effect of silkworm haemolymph on *Escherichia coli β*-galactosidase expression in insect cell lines infected with a recombinant baculovirus. *J. Sericult. Sci. Jpn.* 64:477-481.**

Expression of β-galactosidase (β-Gal) was examined in insect cell lines infected with a recombinant baculovirus, Ac360, in which polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus was replaced by *Escherichia coli* β-Gal gene. Upto 5 cell lines believed to be derived from *Bombyx mori* were examined and a 120 kDa protein corresponding to β-Gal was expressed only in the cell line named S.P.C. Bm 36. The amount of β-Gal expression in infected S.P.C. Bm 36 cells increased with increasing concentration of *B. mori* haemolymph supplemented to the TC-100 medium.

At 2% of the haemolymph, the amount of expressed β-Gal protein accounted for 5.4% of the total cellular proteins, or $7.5 \times 10^3$ units of intracellular β-Gal activity per $5.0 \times 10^5$ cells, which was equivalent to that expressed in the TC-100 medium with 10% fetal bovine serum. It is thus suggested that *B. mori* haemolymph has beneficial effects on the expression of foreign genes by recombinant baculovirus in S.P.C. Bm 36 cells cultured in the TC-100 medium.

**Noguchi Y (1995). Newly isolated cytoplasmic polyhedrosis virus (CPV) derived from *Dendrolimus spectabilis* CPV (DsCPV). *J. Sericult. Sci. Jpn.* 64:493-497.**

A new CPV was isolated fm a larva of *D.s.* infected with DsCPV. It was characterized fm the shape of hexagonal polyhedra observed by light microscopy and from that the field where the viral morphogenesis and polyhedron formation occurred, occupying the central part of virogenic stroma. The newly isolated CPV multiplied in the trachea, muscle and basal granular cells in addition to the midgut cylindrical and goblet cells. This CPV showed almost the same infectivity as the original DsCPV to the larvae of *D. spectabilis*, thus having a comparatively wide host range.

**Okazaki H, Kanaya T, Nishimura S, Ogawa K and Watanabe H (1995). Peroral inoculation of a baculovirus vector to the silkworm, *Bombyx mori*, treated with a low temperature. *J. Sericult. Sci. Jpn.* 64:504-508.**

The baculovirus expression vector system using the sw larvae has been established as useful technology for the production of high value heterologous proteins. In this system, the larvae are inoculated with the vector by intrahemocoelic injection. However, this injection method is not appropriate for a large scale production system as it is time-consuming and labour-intensive. In the present study, we established a peroral inoculation method with a high infection rate, which is applicable to the large scale production system. The method involves feeding the $5^{th}$ instar larvae, which had been chilled immediately after ecdysis, with an artificial diet surface-contaminated with the recombinant BmNPV (BmNPV, 2.3 x $10^6$ PFU/100 µl/g). The optimum conditions for chilling the larvae to induce a high infection rate was the treatment at 2.5 to 5°c for 12 to 24 h. Nearly 100% infection rate was obtained when the larvae were used immediately after chilling. No difference was observed in the degree of expression value of a firefly luciferace gene, inserted into recombinant BmNPV, in the sw b/w intrahemocoelic and peroral inoculation methods.

**Inoue S, Yokota S, Yasanaga C, Funakoshi M, Kawarabata T and Hayasaka S (1995). Continuous culture of *Vairimorpha* sp. NIS M12 (Microsporida : Protozoa) in insect cell lines. *J. Sericult. Sci. Jpn.* 64:515-522.**

Spores of *Vairimorpha* sp. NIS M12 were primed in 0.1 N KOH solution at 27°C for 40 minutes and were mixed with insect cells suspended in Rinaldini's solution. As host cells 4 lepidopteran cell lines (*Antheraea eucalypti*, *Bombyx mori* SES-BoMo-15A, *Spodeptera exigua* Se3FH and *Spodoptera frugiperda* IPLB-Sf21AEII) were used. Low osmolarity of Rinaldini's solution caused a rapid decrease of cell population 24 h post inoculation, and the initial level of cell infection rate was about 7% when a moderate spore/cell ratio of 1:30 was employed. Growth and development of the *Vairimorpha* sp. showed similar patterns in *A. eucalypti* and *S. exigua* Se3FH cell cultures. In *B. mori* SES-BoMo-15A and *S. frugiperda* IPLB-Sf21AE II cell cultures, the growth rate of *Vairimorpha* sp. were lower than those in *A. eucalypti* and *S. exigua* Se3FH cell cultures. After several passages of the infected cell cultures at 27°C, the persistent infection of the *Vairimorpha* sp. was maintained only in the *S. frugiperda* IPLB-Sf21AE II cell line.

## Vol. 65 1996

**Sasaki J, Asano S, Bando H, Lay BW, Hasatowo S, Iizuka T (1996). Identification of *cryV* gene from *Bacillus thuringiensis* isolates. *J. Sericult. Sci. Jpn.* 65:56-61.**

Polymerase chain reaction (PCR) technique and subsequent agarose gel electrophoresis or restriction enzyme digested PCR products enabled us to identify the *cryV* and $cryV_{465}$ genes included in *Bacillus thuringiensis* isolates. At first, PCR using the set of primers which amplified

both *cryV* and *cryV*$_{465}$ genes indicated the presence of at least one of the two *cryV*-type genes in *B. thuringiensis* isolates. Next, agarose gele electrophoresis of *Eco*-RI-digested PCR products revealed which of *cryV* and *cryV*$_{465}$ genes the *B. thuringiensis* isolates contained. Then, *B. thuringiensis* isolates of various serovars were examined and the isolates of serovars *kurstaki*, *sotto* and *aizawai* were found to contain the *cryV* gene. This method was available for searching novel type of *cryV* gene, because many *B. thuringiensis* isolates can be examined rapidly and the variants of *cryV* gene can be detected based on the electrophoretic patterns of the restriction enzyme-digested PCR products.

**Liag FP and Iwashita Y (1996). Healing process of the wound made by puncturing the larval integument of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 65:175-181.**

The abdominal body wall of a 4$^{th}$ instar larva was punctured with a fine needle, and the healing process of the wound was investigated by optical and electron microscopy. The injury caused bleeding and protrusion of various tissues, such as fat bodies, muscles and tracheas. The tissues which protruded through the opening stropped bleeding within 30 min. The wound area turned brownish black due to the oxidation of haemolymph phenol substances. Various hemocytes accumulated around the tissues, haemolymph phenol substances. Various hemocytes accumulated around the tissues, filling the wound opening. The prohemocytes that had accumulated gradually disintegrated and filled the tissue gaps. The granular cells that had gathered over the layer of prohemocytes connected each other with protruding pseudopodia, forming a reticular structure, which finally covered the whole area of the wound opening. On the other hand, the basement membrane of the epidermal cells around the wound opening extended itself and a new cell layer was formed along the extended membrane. At a large wound, two cell layers were formed, the outer one directly enveloping the wound and the inner one connecting itself with the epidermal cells. At the larval molting stage, a new larval cuticle was formed under the wound area, resulting a complete healing of the wound.

**Hashimoto N, Sasaki J, Asano S, Bando H and Iizuki T (1996). *Bacillus thuringiensis* ICP gene expression under the control of *cryIA(a)* gene promotor. *J. Sericult. Sci. Jpn.* 65:185-191.**

A novel expression vector (Phy/IAaP) for *Bacillus thuringiensis* insecticidal crystal protein (ICP) genes was constructed by inserting the *cryIA(a)* gene promoter region into a shuttle vector pHY300PLK, and transducted into Bt51, an acrystalliferous strain. In polyacrylamide gel electrophoresis in the presence of detergent, each of the Bt51 transformants, except for the one with *cryIIA* gene, expressed a peptide of the similar size to that produced in the native strain. Furthermore, electron microscopic observations revealed the similarity in the form of ICP between the transformants and the native strains. Since pHY/IAaP seems to have a single ICP gene expressed in Bt51 under the control of *cryIA(a)* gene promoter, the vector is considered to be useful in the studies of the characteristics of the products of an introduced ICP gene.

**Abe H, Obayashi F, Harada T, Shimada T, Yokoyama T, Kobayashi M and Oshiki T (1996). An application of DNA diagnosis method for preservation of the susceptible strain to densonucleosis virus type-1 of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 65:196-200.**

The nonsusceptibility (complete resistance) to *Bombyx mori* densonucleosis virus type-1 (BmDNV-1) of the silkworm is controlled by a recessive gene, *nsd-1*, in the 21$^{st}$ linkage group. The $+^{nsd-1}$ is a dominant DNV-1 susceptibility gene. We constructed the CSD-1 strain, congenic strain of the C137 (*nsd-1/nsd-1)* for the 21$^{st}$ linkage group, starting with a female of the C137 and a male of the J137 ($+^{nsd-1}/+^{nsd-1}$). Since crossing over is restricted to males in the silkworm, the CSD-1 strain is maintained by repeated backcrossing of the males in the silkworm, the CSD-1 strain contains susceptible (nsd-1/+) and nonsusceptible (*nsd-1/nsd-1*) larvae at a 1:1 ratio. Some

random amplified polymorphic DNAs (RAPDs) in the 21st linkage group of the J137 have been found previously. In order to develop the diagnosis method for the susceptibility of the CSD-1 larvae without virus inoculation and killing, we used these RAPD markers. DNAs extracted from the abdominal legs of 4th instar larvae after ligation, or legs of moths, were individually used as templates for polymerase chain reaction (PCR). We could distinguish the susceptible individuals from the nonsusceptible ones. This diagnosis method is useful for detecting the DNV-1 susceptible individuals without virus inoculation and for obtaining the next generation of diagnosed ones.

**Shi L and Shimizu S (1996). Isolates of protoplasts from *Metarhizium anisopliae* var. *majus*. *J. Sericult. Sci. Jpn.* 65:201-204.**

Conditions for isolation of protoplasts from *Metarhizium anisopliae* var. *majus* were investigated. A high yield of protoplasts from young mycelia of *Metarhizium anisopliae* var. *majus* was obtained by treatment with mixture of Driselase (6 mg/ml), Novozyme (6 mg/ml) and bovine serum albumin (3 mg/ml). sodium chloride was more effective than potassium chloride, sorbitol and glucose as osmotic stabilizers for the preparation of protoplasts.

## Vol. 66 1997

**Sotoshiro H, Ikeda M and Kobayashi M (1997). Identification of a novel virus specific *in vitro* translation product in the midgut of the silkworm, *Bombyx mori*, infected with *B. mori* densovirus type 2. *J. Sericult. Sci. Jpn.* 66:38-47. [English]**

*In vitro* translation of RNA from the midgut of *Bombyx mori* densovirus type-2 (BmDNV-2) infected silkworm during larval-pupal-adult development was performed in a rabbit reticulocyte lysate in the presence of [$^{35}$S] methionine and translation products were analysed by fluorography. The fluorogram showed that not only larval midgut, but also pupal midgut, retained considerable amount of mRNA translatable in the *in vitro* translation system, and the pattern of translation products changed during larval-pupal-adult development and between mock and BmDNV-2 infected midguts. Among the translation products, at least 6 polypeptides with approximate molecular weights (MWs) of 117 K (117,000), 112 K, 45 K, 44 K, 41 K and 18 K were specific to the BmDNV-2 infected midgut. Subsequent immunoprecipitation with anti-BmDNV-2 antiserum revealed that the 45 K, 44 K and 41 K polypeptides were related to polypeptides for BmDNV-2 virion structure. Of these polypeptides, the 41 K polypeptide was not identified among the translation production of the RNA from BmDNV-2 infected silkworm larvae, nor in the purified virions of BmDNV-2. The 41 K polypeptide was a discrete *in vitro* translation product and was most abundant among the BmDNV-2 specific *in vitro* translation products throughout the pupal adult development of the BmDNV-2 infected silkworm. *In vivo* labeling with [$^{35}$S] methionine showed that a polypeptide corresponding in MW to the 41 K polypeptide among *in vitro* translation products was synthesized in the midgut of BmDNV-2 infected pupae.

**Okada E, Mase K, Nagasaka K and Yamamoto T (1997). Difference of inducible antibacterial activity in haemolymph among the silkworm, *Bombyx mori*, races. *J. Sericult. Sci. Jpn.* 66:116-122.**

To elucidate the relationship between the interracial variation in antibacterial resistance of silkworm and its defence mechanisms, the susceptibility of 10 silkworm races was tested against the insect pathogenic bacteria, *Pseudomonas aeruginosa*. The difference in susceptibility was from 40 to 180 fold among the silkworm races. Furthermore, the inducible antibacterial activity of haemolymph was tested by the diffusion method against 2 insect pathogenic bacteria, *P. aeruginosa* and *Serratia marcescens*, and 2 enterobacteria, *Escherichia coli* and *Enterobacter cloacae*. In this assay, all races showed some antibacterial reaction to the enterobacteria and the

activities differed among races. The race Daizo especially showed the highest activities in all test. However, these results of the activity tests did not always correspond with those of the susceptibility tests. The haemolymph induced antibacterial activity was examined by the method of polyacrylamide gel electrophoresis (Native-PAGE). As an antibacterial band in the gel was detected at the same mobility in all races, these differences of the activity are likely to be caused by the quantity of antibacterial substances.

**Hatakeyama Y, Kawakami Y, Iwano H, Inoue T and Ishihara R (1997). Analysis and taxonomic inferences of small subunit ribosomal RNA sequences of five microsporidia pathogenic to the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 66:242-252. [English]**

Nucleotide sequences of small subunit ribosomal RNA (SSUrRNA) of five microsporidia infectious to the silkworm, *Bombyx mori*, are described. Of microsporidia examined, the sequence of SSUrRNA of *Nosema bombycis* SES-NU was very similar to that of *N. bombycis* Sd-NU0IW8201, both having lengths of 1,232 while those of *Nosema* sp. NIS-M11 and *Vairimorpha* Sp. NIS-M12 were 1,247 nucleotides in length and that of *Pleistophora* sp. Sd-NU-IW8201 was 1,248. Similarity of SSUrRNA sequences between *N. bombycis* SES-NU and *N. bombycis* Sd-NU-IW8401 was 99.2%, between NIS-M11 and NIS-M12 96.7% and between *N. bombycis* SES-NU and NIS-M11 85.1%. *Pleistophora* sp. Sd-NU-IW8201 had the least similarity to the other microsporidia examined. SSUrRNA secondary structure models showed that *Nosema* sp. NIS-M11 had the same helices as *Vairimorpha* sp. NIS-M12; however, NIS-M11 was different from *N. bombycis* SES-NU in that it had another helix, suggesting that NIS-M11 was more likely to be a microsporidium of genus *Vairimorpha* than *Nosema*. The secondary structure of SSUrRNA of *Pleistophora* sp. Sd-NU-IW8201 was far different from any other microsporidia examined.

**Yamazaki Y, Okazaki H, Suzuki T, Kanaya T, Ogawa K and Watanabe H (1977). Characteristics of the larva of naked pupa (nd) strains in silkworm, *Bombyx mori* as a host for baculovirus expression vector system. *J. Sericult. Sci. Jpn.* 66:277-281.**

The larva of the naked pupa strain of the silkworm, *Bombyx mori*, fails in spinning due to marked degeneration of the posterior silk gland. It thus becomes a naked pupa without having formed a cocoon. These characters of naked pupa strain are controlled by a dominant gene, *Nd*, on the 25$^{th}$ chromosome. The silk gland is the largest organ in the larva, while it is hardly infected with the nuclear polyhedrosis virus (BmNPV). Accordingly, the superiority of a *Nd* larva to a normal larva with a developed silk gland was examined as a host for the production of foreign proteins in the *B. mori* expression system. Although no difference in body weight was observed between *Nd* and normal (+) 5$^{th}$ instar larvae segregated from back crossing, (*Nd* x+) x+, weights of the anterior and middle portion, and the posterior portion of the silk gland of the *Nd* larvae were 55 and 20%, respectively, of those of the + larva. On the contrary, fat body, one of the tissues most susceptible to BmNPV, was markedly developed in the *Nd* larva, as shown by the 1.6 fold weight of that of the +larva. This was thought to compensate for the degeneration of the silk gland. Moreover, food consumption of the *Nd* larva during the 5$^{th}$ instar was less than 90% of that of the +larva. Expression of a firefly luciferase gene in the baculovirus expression system indicated that the level of expression in the *Nd* larva was 20-25% higher than in the +larva. These results support the belief that a *Nd* larva is an economically efficient host for the *B.mori* baculovirus expression system.

**Sugimura Y, Uehara H, Kotani E and Furusawa T (1997). Evaluation of polysaccharides as elicitor inducing antibacterial activity in larvae of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 66:285-287.**

Various immune responses are induced in the silkworm (*Bombyx mori*) only after inoculation of bacteria and administration of bacterial cell components. One drastic response is

the *de novo* synthesis of antibacterial proteins, when bacterial components such as peptidoglucan and lipopolysaccharides LPS) are applied. Another humoral response is the melanosis of haemolymph, which is induced by β-1,3 glucan via the operation of the propolyphenoloxides cascade system. Antibacterial activity induced in haemolymph of silkworm larvae by application of various polysaccharides is described.

**Iizuka T (1997). Insecticidal crystal protein from *Bacillus thruringiensis* and its mode of action. *J. Sericult. Sci. Jpn.* 66:311-322. [Japanese]**

**Shimizu S and Yoshioka T (1997). Factors which affect the production of extracellular protease in the entomopathogenic fungus *Paecilomyces fumosoroseus*. *J. Sericult. Sci. Jpn.* 66:357-359. [Japanese]**

**Iguchi T, Iwano H, Hatakeyama Y, Kawakami Y, Onoda K, Hayasaka S, Inoue T and Ishihara R (1997). Sporogony of a microsporidium, *Nosema* sp. NIS-M11 (Microspora: Nosematidae) in larvae of the silkworm, *Bombyx mori raised* under two distinct levels of temperature. *J. Sericult. Sci. Jpn.* 66:445-452.**

Modes of spore formation in the larvae of silkworm, *Bombyx mori* of a microsporidium, *Nosema* sp. NIS-M11 9FUJIWARA, 1980) was investigated. Larvae of the silkworm, after inoculation of *Nosema* sp. NIS-M11 were raised at 26°C or 18°C. Light and electron microscopical observations revealed that two types of spore were formed in muscle and fat body close to the midgut. The one type was formed through the apansporoblastic (*Nosema* like) pattern of development of sporogony, while the other type of spore was formed through the pansporoblastic (*Thelohania* like) pattern of development of sporogony. *Nosema* type spores dominted in muscle and fat body from silkworm raised at 26°C, while *thelohania* type spores became dominant in muscle and fat body of silkworms raised at 18°C. Sporophorous vesicles having uninucleated spores, harbored numerous electron dense granules and electron lucent tubular materials which are reported to be characteristic to *Vairimorpha necatrix* (MITCHELL and CALI, 1993). These observations suggest that *Nosema* sp. NIS-M11 is not a microsporidium of genus *Nosema*, but a *Vairimorpha* sp.

**Kageyasu S, Hayakawa T, Isawa H, Asano S, Sahara K, Iizuka T and Bando H (1997). Detection of the silkworm-pathogenic virus genomes by PCR. *J. Sericult. Sci. Jpn.* 66:477-483.**

The five viruses, *Bombyx mori* densonucleosis virus type-1 (BmDNV-1), type-2 (BmDNV-2), *Bombyx mori* nuclear polyhedrosis virus (BmNPV), *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV) and Infectious flacherie virus (IFV) are the agents causing fatal diseases of silkworm. The aim of this study is to establish a simply and highly sensitive PCR system for detection of all the viruses. A combination of the RT-PCR and the nested PCR was found to be effective to amplify DNA and/or RNA virus genome sequences in a reaction maintaining high sensitivity and specificity. In this report, the primer design and the condition of the PCR for the detection of all the viruses are described.

## Vol. 67 1998

**Nagata M and Sun PJ (1998). Multiplication of nuclear polyhedrosis virus in the silkworm larvae during the latter period of the 5$^{th}$ instar. *J. Sericult. Sci. Jpn.* 67:23-29.**

The concentrations of nuclear polyhedrosis virus (NPV) in the haemolymph after injection of the virus wwere examined immunologically at various ages of the 5$^{th}$ larval instar of the silkworm, *Bombyx mori* and multiplication curves of the virus were compared. Multiplication rates of the virus at logarithmic phase were almost constant during the larval ages and some

differences in the virus multiplication among larval ages were inferred to be in the initial phase of the virus multiplication. Although all larvae infected with NPV were finally died, mortality at 7 days after infection varied with larval ages, lowest at day 4 and increase towards end of the instar. Spinning and pupation were delayed with virus infection and this interference of the virus with silkworm development induced its death in the cocoons.

**Matsuki N, Mitamura T and Doi N (1998). Effect of wood vinegar to fungus disease of silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 67:143-145.**

Wood vineager is useful for the control of plant pathogenic fungi against sapling of needle leaf tree. We examined effect of wood vinegar to fungus disease of silkworm.

Wood vinegar made from mulberry branches and marketing wood vinegar named Junseimokusakueki were used for the examination. Two types of wood vinegar checked germination of conidium and restrained growth of hypha of *Beauveria bassiana*. Spray of wood vinegar to rearing bed restrained fungus disease of silkworm, and didn't influence quantitative character of silkworm. Wood vinegar oshould be useful for the control of fungus disease of silkworm.

**Yamakawa M (1998). Insect antibacterial proteins: Regulatory mechanism of their synthesis and a possibility as new antibiotics. *J. Sericult. Sci. Jpn.* 67:163-182. [Japanese-Review]**

**Sakurai M, Shikata M, Sano Y, Hashimoto Y and Matsumoto T (1998). Virulence of *Autographa californica* nucleopolyhedrovirus infection of non-permissive cultured cells of the silkworm, *Bombyx mori*. *J. Sericult. Sci. Jpn.* 67:211-216. [English]**

Virulence of *Autographa californica* nucleopolyhedrovirus (AcMNPV) infection against a non-permissive silkworm cell line (BmN4) was examined. AcMNPV was added at different doses to BmN4 cells, and threshold dose of virus inoculum which determined fate of cells in culture was between $TCID_{50}$/cell of 1.4 and 0.7. This threshold virus dose was very close to one infectious virus particle per cell. Cell growth was completely inhibited in cultures infected at more than threshold virus level. Viral DNA accumulation in infected cells was less than that in BmN4 cells infected with *Bombyx mori* NPV (BmNPV). Since no production of budded virus (BV) was detected in cells transfected with AcMNPV DNA, it is likely that the BV detected in cultures of AcMNPV infected silkworm cells during incubation was not progeny virus, but was carried over from inoculum.

**Asano SI, Pujiastuti Y, Sahara K, Bando H, Kukuta H and Iizuka T (1998). Identification of *cry-1* genes from *Bacillus thuringiensis* strains which have activity against *Spodoptera lutura*. *J. Sericult. Sci. Jpn.* 67:237-242.**

*Bacillus thuringiensis* reference strains and isolates used in this experiment were stored in our library and originally isolated from soil and dead insects in Hokkaido. In order to find *B. thuringiensis* strains which have a high toxic activity against *Spodoptera litura* larvae, these *B. thuringiensis* strains were bioassayed to the 3$^{rd}$ instar larvae of *S. litura*.

The identification of *cry1* genes from active strains against *S. litura* larvae were demonstrated by using PCR. *Cry1C* cloned from serovar *entomocidus* and servovar *kenyae* revealed a toxic activity against *S. litura* larvae. On the contrary, *cry1E* gene cloned from serovar *tolworthi* and serovar *darmstadiensis* did not have a toxic activity.

On the other hand, even though serovar *galleriae* Acp10-8 and *wuhanensis* have a highly toxic activity against*S. litura* larvae, both strains did not involve *cry1C* and *cry1E* genes. It seems that they have the novel *cry* genes.

**Miyajima S, Mori A, Hagiwara K, Kobayashi J, Yoshimura T, Nakai K, Suzuki Y and Tomita M (1998). Microheterogeneity in the polyhedrin gene of *Bombyx mori*, a cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Jpn.* 67:287-294. [English]**

Using reverse transcription and the polymerase chain reaction, ten positive clones harbouring the polyhedrin gene were isolated from H and I strains of the *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV). Eight differences at the point mutation level were detected between the both strains in nucleotide sequences of the cloned polyhedrin genes. Four of these at nucleotide positions 286, 436, 562 and 639 respectively resulted in changes in amino acid residues 82, 132, 174 and 200 of polyhedrin. Those at positions 82 and 132 where valine residues in H strain were replaced with glutamic acid in the I strain appeared to be of particular significance. Regular hexahedral occlusion bodies were formed on expression of the cloned polyhedrin gene of the H strain in a baculovirus system, whereas the expression of that from BmCPV strain I did not result in the regular icosahedral polyhedra generally observed. Some gene products seemed to be needed for the formation of the icosahedral polyhedra. The results indicate that microheterogeneity exists in the polyhedrin gene between BmCPV strains H and I, resulting in marked changes in amino acid residues at 82 and 132 with some influence on the configuration and control of occlusion body shape.

**Eguchi R, Hara W, Shimazaki A, Hirota K, Ichiba M, Ninagi O and Nagayasu KI (1998). Breeding of the silkworm race "Taisei" non-susceptible to a densonucleosis virus type 1. *J. Sericult. Sci. Jpn.* 67:361-366. [English]**

The No.908 strain of the silkworm, *Bombyx mori* has a dominant gene, *Nid-1*, in bomozygous, which controls the non-susceptibility of the silkworm to densonucleosis virus type 1 (DNV-1). To establish a silkworm race, non-susceptible to DNV-1, the *Nid-1* gene was introduced to a practical race, N 150.The F1 hybrid of N 150 x No.908 was recurrently backcrossed with N 150 for severl generations and individuals having heterozygous *Nid-1* (*Nid-1/+*) were ascertained in every generation as survivors of the oral inoculation of DNV-1. In the 6$^{th}$ generation of back crossing, *Nid-1/+* individuals were sib mated to establish a strain having homozygous *Nid-1*. In the subsequent generation, the progeny was exposed to DNV-1, and the survivors, whose genotype was supposed to be *Nid-1/Nid-1* or *Nid-1/+*, were further sib mated. Some males which had been used in the sib mating were also mated with female of the susceptible race to test whether the genotype of the male is homozygous *Nid-1* or not. In the 2$^{nd}$ generation of the sib mating with males which had been ascertained to have homozygous *Nid-1*, a progeny line produced all of batches containing only non-susceptible larvae was determined to be the strain with homozygous *Nid-1*. After improvement in economic characters for several generations, the strain with homozygous *Nid-1* was established as the practical race, N203, non susceptible to DNV-1. The cross N203 x C150 was authorized by the Minister of Agriculture, Forestry and Fisheries in March 1996, as the commercial silkworm race named "Taisei" for spring rearing.

**Ono K and Iwashita Y (1998). Histo- and cyto-pathological changes induced by *Bacullus thuringiensis* δ-endotoxin in the midgut epithelium of silkworm larvae. *J. Sericult. Sci. Jpn.* 67:453-459.**

Histo- and cyto-pathological changes induced by *Bacullus thuringiensis* δ-endotoxin in the midgut epithelium of silkworm larvae were observed. Primary damage was demonstrated in anterior midgut epithelium cells within 30 minutes subsequent to the inoculation of *B. thuringiensis* toxins. In columnar cells, microvilli were deformed and disrupted. Mitochondria and endoplasmic reticulum in the cytoplasm were morphologically changed and collapsed. After the cytoplasm had swollen and electron transarent was recognized, cytoplasmic protrusions were revealed to extrude from the cell surface. These protrusions finally burst and dropped into the lumen. In goblent cells, the cytoplasm degenerated and the shape of microvilli changed and then

disappeared. Mitochondria in the microvilli entered the cytoplasm, degenerated and disappeared. When the cytoplasm degenerated, the cells gradually decreased in volume and contracted to assume spherical shape on the basement membrane. Damages of columnar and goblet cells was also apparent at the same time and the rate of morphological changes was the same in all cases. It was found that loss of ion regulation in goblet cells was the cause of death of intoxicated larvae.

**Wada S and Shimizu S (1998). Isolation of protoplasts from an entomopathogenic fungus *Beauveria brongniartii*. *J. Sericult. Sci. Jpn.* 67:499-502. [English]**

Conditions of the isolation of protoplasts from an entomopathogenic fungus, *Beauveria brongniartii* were investigated using two strains. A high yield of protoplasts was obtained from mycelia in both strains by treatment with the cell wall lytic enzyme, Novozyme 234, after incubation for 1 h at 30°C. Sodium chloride and potassium chloride were more effective as osmotic stabilizers for the isolation of protoplasts than sorbitol, mannitol or glucose. There were some differences in optimal conditions of protoplast isolation between strains.

## Vol. 68 1999

**Tsuda H, Nakagaki M, Hashimoto T, Kajiura Z, Takei R and Iwashita Y (1999). Multiplication of NPV and expression of polyhedrin gene in the midgut epithelium of silkworm *Bombyx mori*. *J. Sericult. Sci. Jpn.* 68:19-25.**

We determined the amounts of viral DNA, polyhedrin mRNA and polyhedrin in the midgut epithelial cells infected with BmNPV, in order to study the poor production of polyhedrin in these cells. When the appearance and accumulation of polyhedrin in mRNA in the midgut epithelial cells were investigated by northern blot analysis, polyhedrin mRNA were detected as early as 6 h post inoculation (pi). At 12 h pi, amount of polyhedrin mRNA increased in the midgut epithelial cells. Quantitative PCR of virus DNA in the midgut epithelial and fat body cells at 72 pi indicated that amount of the virus DNA in midgut epithelial cells was close to that in fat body cells, suggesting that virus DNA synthesis occurred in midgut epithelial cells. Quantitative RT-PCR for polyhedrin mRNAs in the midgut epithelial and fat body cells at 72 h pi indicated that amounts of the mRNA in both cells did not differ significantly each other, suggesting that the transcription of polyhedrin gene occurred markedly in midgut epithelial cells. Western blot analysis showed that polyhedrin was produced in midgut epithelial cells, but its amount was much lower than that in fat body cells. It is concluded that low level translation of polyhedrin gene may be related to poor polyhedron formation in midgut epithelial cells.

**Nakagaki M, Morinaga T, Zhou CQ, Kajiura Z and Takei R (1999). Increasing curves of two virus DNAs in the midgut epithelium of silkworm infected with *Bombyx mori* densonucleosis virus type 2 (BmDNV-2). *J. Sericult. Sci. Jpn.* 68:173-180.**

Increasing curves of 6.1 nd 6.6 kb DNAs of *Bombyx mori* densonucleosis virus type 2 (BmDNV-2) in the midgut epithelium infected with BmDNV-2 were investigated using a quantitative competitive PCR technique. The 6.1 and 6.6 kb DNAs in midgut epithelium increased logarithmically with time, reaching a maximum titer of 500,000 fold at 48 h since the infection (h post infection, hpi). The maximum titer was maintained until 102 hpi after reaching the maximum. Two DNAs during larval ecdysis decreased to 1/625 of maximum titer, indicating that virus infected cells have dropped out of midgut epithelium. Since increasing curves of two DNAs were very similar, each of two DNAs could be used as template DNA for BmDNV-2 detection by PCR. In susceptible race of the silkworm to BmDNV-2 disease, two virus DNAs increased until 12 hpi and then decreased rapidly, suggesting that some of viral DNAs have replicated and accumulated in midgut of the insusceptible race. The replication of 6.1 kb DNA went ahead of that of 6.6 kb DNA in sensitive and insusceptible races of the silkworm. It is suggesting that two DNAs could replicate independently.

**Pujiastuti Y, Asano SI, Sahara K, Bando H and Iizuka T (1999). Toxicity of *Bacillus thuringiensis* subsp. *wuhanensis* crystal protein to *Bombyx mori* and *Spodoptera litura*. *J. Sericult. Sci. Jpn.* 68:195-199. [English]**

*B. thuringiensis* subsp. *wuhanensis* contains three types of *cry1* genes: *cry1Ab*, *cry1Ac* and *cry1D*. Specific *cry1Db* primers were designed to clone the *cry1Dbw* gene. The DNA of the *cry1Dbw* gene sequence exactly matched the *cry1Db* gene sequence proposed by LAMBERT (1993). We isolated three types of crystal protein, Cry1Ab, Cry1Dbw and w*uhanensis* from this strain. The cry1Dbw and *wuhanensis* proteins showed highly toxicities against the silkworm larvae, *Bombyx mori* and the common cutworm, *Spodoptera litura*. On the other hand, the Cry1Ab protein was not toxic to *B. mori* and showed low toxicity to *S. litura*. We reveal that their toxicities are caused by the *cry1Dbw* gene, and propose that this gene is very important for controlling the pest insects.

**Zhou CQ, Nakagaki M, Takizawa K, Tsuda H, Kajiura Z, Takei R and Iwashita Y (1999). Inactivation of *Bombyx mori* nucleopolyhedrovirus (BmNPV) with calcium hydroxide solution. *J. Sericult. Sci. Jpn.* 68:201-207.**

When the nuclear polyhedra were exposed to calcium hydroxide solution, polyhedra and virus structural proteins were degenerated into proteins with the lower molecular weight. The process of virion dissolution in the calcium hydroxide solution was observed by the electron microscope. At first, the nucleocapsids protruded from an opening on the viral envelops. The protruded capsids were dissolved and then the remaining envelops were dissolved. Calcium hydroxide solution degraded the genomic DNA of nucleopolyhedrovirus (BmNPV) into fragmented DNAs with the lower molecular weight. In this paper, we have demonstrated that inactivation of BmNPV with calcium hydroxide solution results from not only blocking of viral attachment on and its penetration into cells, but also the degrading of the infectious virus DNA.

**Kikuta H, Kuroiwa M, Takagi R and Iizuka T (1999). Study on the isolation and identification of *Bacillus thruringiensis* in soil samples from Yakushima Island (I) Serological identification and flora. *J. Sericult. Sci. Jpn.* 68:217-223.**

Yakushima Island is located south of Kyushu Island. Nature is well preserved on this island. Since *Bacillus thuringiensis* strains have been effectively isolated in soils from southern Asia, we tried to isolate *Bacillus thuringiensis* and identify H-serotype. Based on these results, the flora of *Bacillus thuringiensis* is discussed in this study.

1. Soil samples from 177 spots in 35 sections of Yakushima Island were investigated and 3955 strains belonging to *Bacillus cereus* from 68,153 spore forming bacterial strains were microscopically determined. Lastly, 53 *Bacillus thuringiensis* isolates were serologically investigated.
2. Based on the H-serotyping results, 24 strains of serovar *kurstaki*, 14 strains of serovar *galleriae* and 6 strains of serovar *sumiyoshiensis* were identified as dominant strains. This result indicates that the *Bacillus thuringiensis* flora in Yakushima Island is relatively unique.

**Kikuta H, Kuroiwa M, Takagi R and Iizuka T (1999). Study on the isolation and identification of *Bacillus thruringiensis* in soil samples from Yakushima Island (II) Insecticidal activity and the identification of *cry* genes. *J. Sericult. Sci. Jpn.* 68:225-235.**

We previously reported that 53 *Bacillus thuringiensis* isolates from Yakushima Island were serologically investigated. In the present study, the insecticidal activity of these strains against the silkworm *Bombyx mori* and the mosquito larvae *Aedes japonicus* was investigated and the identification of *cry* genese in these strains was conducted using the PCR method.

Oligonucleotide primers used for making DNA probes were *cry1Aa, cry1Ab, cry1Ac, cry1Ba, cry1Ca, cry1Da, cry1ea, cry2Aa, cry4Aa, cry1Ba, cry10Aa* and *cry11Aa* genes depending on their specific nucleotide domains. *Cry* gene profiles of 24 serovar *kurstaki* strain were the same for *cry1Aa, cry1Ab and Cry1Ac* with a type strain of HD-1 except for *cry1Aa* the Jan09-2-2 strain composed of *cry1Aa* and *cry1Ab.* All of the strains included the *cry2Aa* gene.

In the 14 strains of serovar *galleriae*, all of the strains included the same *cry1Ab* and *cry2Aa* genes as the type strain. In the other serovar from Yakushima Island, *thuringiensis kenyae* and *israelensis* strains displayed different profiles from the type strain used in this experiment. Serovar *kurstaki* Jano9-2-2 revealed high insecticidal activity against silkworm larvae, compared with the control strain HD-1. Serovar *Israelensis* Aiko2-1-1 also revealed high activity against the *Aedus japonicus* larvae compared with the type strain. These two strains seem to include novel *cry* genes.

**Matsumoto N (1999). Ihibition effect of propionates on the insecticidal activity of *Bascillus thruringiensis* (Bt) formulations. *J. Sericult. Sci. Jpn.* 68:333-338.**

BT (*Bacillus thuringiensis*) formulations are one of the most commonly used microbial insecticides. The insecticidal activity of the BT formulations has been examined according to the standard bioassay method constructed by the Agricultural Chemicals Inspection Station (ACIS) in Japan, which is an “oral inoculation method” using BT containing artificial diets and silkworm larvae. Propionates (propionic acid, calcium propionate and sodium propionate, etc) fatty acid antiseptics of foods, should be useful for antiseptics of artificial diets of the silkworm. However, there has not been reported on the effect on the BT activity of propionates added in diets.

In the present study we showed that there was not a significant difference between propionates treated and untreated silkworm larvae in the diet consumption and the larval weight inhibitions (P≤5% level by Dankan’s multiple range test). Nevertheless larval mortality dropped significantly in the diets with 0.02 to to 2% calcium propionates (CaP 0.02 to 2%). In particularly, when larvae were reared with the CaP 0.06% diet (CaP was added to the ACIS standard diet) over the tested period, the BT ($LC_{50}$) mortality was reduced more than 5.1 fold compared to that of the same diet without CaP. In conclusion, it was strongly suggested that the propionates act as an antidote against the BT toxin, when added to the artificial diet as an antiseptic.

## Vol. 69 2000

**Komoto N, Thibert C, Boulo V, Fang Q, Burns JC and Couble P (2000). Gene introduction into silkworm embryos with a pseudotyped retroviral vector. *J. Sericult. Sci. Jpn.* 69:55-61. [English]**

Gene transfer systems for producing transgenic animals have been investigated using different types of vectors. In this report, we tested pseudotyped retroviral particles for their ability to transfer genes into *Bombyx mori*. Silkworm eggs were injected with pseudotyped retroviral vectors derived from the Moloney murine leukemia virus carrying the green fluorescent protein coding sequence inserted under the control of a long terminal repeat promoter. PCR analysis and Southern hybridization showed that the vector particles successfully infected *Bombyx* embryo cells and that the viral genome was reverse transcribed and amplified during development. An inverse PCR assay showed the integration of the vector sequence into the silkworm genome.

**Wu FQ, Lavina BA, Ikeda M, Shirata N, Cai YX, Pan SX and Kobayashi M (2000). Cloning and biological characterization of *Spodoptera exigua* nucleopolyhedroviruses isolated from China. *J. Sericult. Sci. Jpn.* 69:177-189.**

Fifteen necleopolyherdrovirus (NPV) clones designated as SeNPV G1-G15 were derived by plaque assay from the uncloned NPV population that was isolated in China from the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). Restriction endonuclease analysis of genomic DNA showed that these 15 clones fell into three groups represented by G1, G3, and G4. The genomic DNAs of G1, G3 and G4 showed a similar overall restriction endonuclease pattern and a polymorphism in the size and number of restriction endonuclease fragments. Examination with seven different lepidopteran insect cell lines derived from *Bombyx mori* (BmN-4), *Lymantria dispar* (IPLB-Ld652Y), *Spodoptera exigua* (Se301), *S. frugipera* (Sf9), *S. ittoralis* (CLS-79), *S. lutura* (tuat-sPlI221), and *Spilosoma imparilis* (FRI-SpIm-1229) showed that Se301, Sf9 and FRI-SpIm-1229 cell lines were permissive for all the SeNPV clones obtained, in which only the Se301 cell line was able to support a high titre of SeNPV replication. Comparative biological characterization in Se301 cells showed that among the SeNPV clones, G3 had the highest potential for the production of viral DNA, budded virions, and polyhedrin in the infected cells. All the SeNPV clones caused an apoptoric-like cytopathology in a significant proportion of IPLB-Ld652y AND fri-SpIm-1229 cells, and a maximum degree of the apoptotic like cytopathology was observed in the cells infected with G3. These results indicate that the SeNPV clones obtained in the present study are closely related clonal variants that exhibit different biological activity due to minor genomic differences.

**Imai N, Ali SE, El-Singaby NR, Iwanaga M, Matsumoto S, Iwabuchi K and Maeda S (2000). Insecticidal effects of a recombinant baculovirus expressing scorpion toxin LqhIT2. *J. Sericult. Sci. Jpn.* 69:197-205. [English]**

A system of the baculoviruses *Bombyx mori* nucleopolyhedrosis virus (BmNPV and the silkworm larvae, *Bombyx mori* was applied to analyze the potential use of the insect specific toxin LqhIT2 for improving the efficiency of recombinant baculoviruses for pest control. The LqhIT2 gene, along with the signal sequence for secretion was synthesized and transferred into the BmNPV genome to generate the recombinant virus BmLqhIT2. Larvae infected with BmLqhIT2 showed LqhIT2 specific symptoms at 48 h post injection (pi), stopped feeding at 60 hpi and became motionless by 84 hpi. Particularly, the larvae infected with BmLqhIT2 stopped moving 24 h earlier than those infected with BmAaIT, which expressed another insect specifica scorpion toxin AaIT. An N-terminal sequence and mass spectrometric analysis showd that BmLqhIT2 produced mature LqhIT2. the present results suggest that LqhIT2 is more efficient than AaIT for improving insecticidal activity of baculovirus.

**Yamagishi J, Asano S, Sahara K, Iizuka T and Bando H (2000). PfDNV nonstructural proteins suppress viral RNA splicing. *J. Sericult. Sci. Jpn.* 69:271-276. [English]**

Alternative splicing observed in the mRNAs for the structural proteins of PfDNV seems to be necessary to generate five structural proteins from two separated ORFs. Involvement of several splicing donor sites and acceptor sites in the alternative splicing complicates understanding of the splicing mechanism of the PfDNV. First we developed a method for detection of spliced RNA molecules using the combination of the different two techniques. RT-PCR and primer extension. This splicing-detection method demonstrated that the sites of alternative splicing occurred within the PfDNV RNA was also recognized in S2 cells (a *Drosophila* cell line). Interestingly, a drastic suppression of the splicing and the accumulation of the unspliced RNA molecules were observed in S2 cells transfected with the PfDNV non-structural proteins ($\gamma$ and $\beta$)-expression plasmids. These results suggested that the cellular factors play an important role on the selection of the specific splicing sites and that the viral nonstructural proteins may regulate it in a suppressive manner.

## Vol. 70 2001

**Kawakami Y, Iwano H, Hatakeyama Y, Inoue T, Canning EU and Ishihara R (2001). Use of PCR with the specific primers for discrimination of *Nosema bombycis*. *J. Sericult. Sci. Jpn.* 70:43-48. [English]**

The specific amplification by PCR using a pair of primers, KAI01 and KAI02 was reinvestigated as to its ability to distinguish microsporidia, belonging to genus *Vairimorpha*, *Pleistophora* and *Nosema*. DNA samples from microsporidia of genus *Vairimorpha* or *Pleistophora* so tested, three species of *Vairimorpha* and one *Pleistophora*, failed to produce PCR products by those primers. Of *Nosema* spp., *N. apis*, *N. furnacalis*, *N. mylittensis* and *Nosema tyriae* from cinnabar moth, *tyria jocobeae* did not also produce any product. Microsporidian parasites isolated from silkworms infected with pebrine in the northern part of India and in southern India and in china gave PCR products by KAI01 and KAI02 primers. The results from our study suggest that PCR tests with use of KAI01 and KAI02 primers are useful to distinguish highly virulent strains of *N. bombycis* in pebrine inspection.

**Kojima K, Hirano A, Asano S, Sahara K and Bando H (2001). Analysis of the BmDNV-2 specific DNA polymerase and the common terminal sequence binding proteins. *J. Sericult. Sci. Jpn.* 70:103-108.**

Recent studies of BmDNV-2 predicted a unique replication mechanism different from the parvoviruses and implied that the common terminal sequence (CTS) of 53 nucleotides and the viral structural protein with the DNA polymerase motif (p128) played important roles in the viral DNA replication. To understand the replication mechanism of BmDNV-2, the function of the CTS and the properties of the viral DNA polymerase were studied. The gel mobility shift assay revealed that at least one of the viral structural proteins bond to the 3'-CTS, emphasizing the property of the idea that the CTS was a putative replication origin of the viral DNA. On the other hand, an increase of the DNA polymerase activity with characteristic properties was observed in the midgut of the sw infected with BmDNV-2. These observations suggest that the farther characterization of the BmDNV-2 specific DNA polymerase is indispensable to understanding the replication mechanism of BmDNV-2.

**Hatakeyama Y and Hayasaka S (2001). Specific amplification of microsporidia DNA fragments using multiprimer PCR. *J. Sericult. Sci. Jpn.* 70:163-166. [Japanese]**

**Yazawa C and Shimizu S (2001). Comparison of the nucleotide sequences of elastase-like serine protease genes from *Metarhizium anisopliae*. *J. Sericult. Sci. Jpn.* 70:167-170. [Japanese]**

## Vol. 71 2002

**Arakawa T, Furuta Y, Kubomura Y, Kato M and Hayasaka S (2002). Peroral infection of nucleo poly-hedrovirus non-occluded viral particle of *Bombx mori* L. aided by a fluorescent brightener. *J. Sericult. Sci. Jpn.* 71:101-105.**

A procedure to perorally infect the sw with NPV non-occluded viral particles was developed. The sw larvae were fed an artificial diet containing Tinopal UNPA-GX(Tinopal), a fluorescent brightener, for 24 h, then fed a diet contaminated with the non-occluded viral particle of BmNPV. A haemolymph supernatant of sw larvae infected with BmNPV from which the viral polyhera had been removed was used as an original inoculum. When the sw larvae were fed a diet with 0.3% (w/w) Tinopal (1400g/1000 larvae) and administered an inoculum that was

diluted one-thousand times (50 ml/600 g diet/1000 larvae), all the larvae died of viral infection. A diet containing 0.3% Tinopal which had been autoclaved (121°C, 20 min) and stored at 5°c for 300 days did not lose its activity to enhance viral infection. This newly developed peroral inoculation method of non-occluded BmNPV particle utilizing Tinopal will lead to industrial pharmaceutics production for using a baculovirus vector in a huge number of insect hosts.

**Vol. 72 2003**

**Arakawa T and Kozuma K (2003). Peroral infection of baculovirus vector in the prefinal instar larvae of *Bombyx mori* aided by flufenoxuron, an insect growth regulator. *J. Sericult. Sci. Jpn.* 72:15-18.**

Recombinant human serum albumin (rHSA) was produced in the sw by peroral infection of a baculovirus vector carrying an HAS gene aided by flufenoxuron. Flufenoxuron, an insect growth regulator, was dissolved in acetone and added to an artificial diet resulting in the final concentration of 0.1% flufenoxuron and 2% acetone in the diet. Newly ecdysed 4$^{th}$ instar larve of sw were fed the diet containing clufenoxuron for 21 h. Thereupon the larvae were inoculated with a baculovirus vector carrying and HAS gene perorally at 2.5 x $10^6$ $TCID_{50}$ units/larva after they had been starved for 3h. The inoculated larvae were reared at 26°C on an artificial diet. rHSA was detected in the whole body homogenate of the inoculated larvae at 307±33.9 µg/larva (average ±SD) 4 days after inoculation. Although the weight gain of larvae administered flufenoxuron was retarded in 1 day, the final weight was similar to that of the control larvae.

**Nagata M, Nakao R, Hamada K and Aoki F (2003). Inactivation of *Bombyx mori* nucleopolyhedrovirus by reducing agents. *J. Sericult. Sci. Jpn.* 72:49-54.**

Reducing agents such as glutathione and ascorbic acid inactivated a nucleopolyhedrovirus (NPV) of the silkworm *in vitro*. Activity of BmNPV virions was lost after incubation with 5mM reduced glutathione or 5mM ascorbic acid at 25°C for 1 day. A half time of inactivation at 0.05% (2.8 mM) of ascorbic acids was estimated to be 1.5 min. NPV in the haemolymph from NPV-infected larvae was inactivated after treatment with 1% ascorbic acid at 25°C for 1 day. From these results, applications of ascorbic acid to disinfection against sw viruses were discussed.

**Vol. 73 2004**

Nil

# Author Index