Virus-insect relationships

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Preface

The study of virology as a whole is now so much in the hands of the biochemist and the molecular biologist that the reactions of the organism which is the host of the virus tend to be overlooked.

This book has been written by one of the few remaining old-fashioned virologists who has not quite lost touch with the living organism and its interesting relationships with the virus. It is hoped therefore, that there is still scope for a book on insect virology written from a mainly biological point of view. In consequence detailed accounts of the chemical nature of the viruses and their molecular biology have been omitted and left to those with the necessary specialized knowledge to fill the gap.

Insect virology is a young discipline: the study of insect viruses was actually started for the first time in the United Kingdom by myself in 1950. In consequence there is no extensive background of research, such as exists in the older virological disciplines, on which to base conclusions. This restricts to some degree a fully critical presentation of the subject. However, there is no reason to suppose that the standard of research on insect virology is any lower than that pertaining to other branches of virology.

The use of the terms 'inclusion' and 'non-inclusion' types of diseases has been criticized as obsolete by some exponents of virus nomenclature and classification. Nevertheless I have employed these terms in this book because they offer a natural and easy means of differentiating the two large groups of insect viruses.

Grateful acknowledgement is due to Council of the Royal Society for a grant of money towards the expenses of writing this book. I am also greatly indebted to many friends and colleagues who lent me photographic prints or sent me reprints; the names of these authors are given under each borrowed illustration. I would mention especially Dr Robert R. Granados, Dr Basil Kassanis, FRS, and Dr Max Summers for their kind assistance in various ways. I am grateful also to Dr T. W. Tinsley and his colleagues for kindly criticizing certain chapters and for making helpful suggestions, but any errors or omissions are, however, my responsibility. Mr Robert Welham, Science Publisher, and Miss Bobbi Gouge, Longman Group, have been most helpful throughout. Finally I would like to thank Mrs Doreen Piggott for her help in typing this book.

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Introduction

The first part of this book is devoted to an account of the various kinds of virus diseases affecting insects together with the causative viruses.

The characteristics of the virus diseases, or group of virus diseases, are described first in general terms to give the reader an idea of the symptom picture presented by a particular disease. There follows a more detailed description of one or more specific viruses, and the diseases caused, chosen as representative of the group.

It is obviously impossible to give detailed accounts of the many hundreds of virus infections of insects. The descriptions of specific diseases follow a common plan throughout. First, the inclusion body, if present, is described; this is followed by a detailed account of the causative virus particle and its properties so far as they have been ascertained, shape, ultrastructure, etc., mode of replication, disease caused, purification methods, serology, transmission, host range (if any) and geographical distribution. Some features, such as serology and virus replication, are only touched upon in the descriptions of individual virus infections since these are dealt with in more detail later in the book.

In the rest of the book matters of more general application in insect virology are discussed, including the important subject of the part played by insects and other arthropods in the spread of plant and animal viruses and the use of viruses in the biological control of insect pests.

Contents

Part 1

The different types of insect virus diseases

A.

The inclusion-type diseases

This type of disease is characterized by the development of intracellular inclusions. The polyhedroses contain the nuclear and cytoplasmic polyhedral crystals, the granuloses contain the crystalline capsules, and the entomopox diseases contain the spherules and spindles. With the exception of the spindles, the virus particles are occluded in the crystals.

Chapter 1

The polyhedroses — Baculoviruses — Nuclear Polyhedroses (NPV)

The viruses of the NPs are DNA-containing, the percentage varying slightly in the different diseases.

The chief characteristic of the nuclear polyhedroses, as also of the cytoplasmic polyhedroses, granuloses and entomopoxviruses, is the presence in the tissues of the affected larvae of vast numbers of polyhedral inclusion bodies which are actually small protein crystals (see Fig. 1.14). The virus particles themselves, which in the nuclear type of disease are invariably rod-shaped, are occluded in the crystals. It is noteworthy that no other body or cell organelle is ever found also occluded.

The polyhedral bodies occur in different shapes and sizes and may vary in different insects or even in the same insect but tend to be of the same size in the same cell. In the silkworm, ***Bombyx mori***, the prevailing shape is that of a dodecahedron whereas in ***Lymantria monacha*** the inclusions are mainly tetrahedra. In ***Hyphantria cunea*** two types of nuclear polyhedra may occur, hexahedra and tetrahedra, while those from ***Porthetria dispar*** are irregular in shape. The size varies from 0.5–15 μ according to species.

In certain species the polyhedra have a characteristic appearance; in the larvae of the scarlet tiger moth ***Panaxia dominula***, for example, the polyhedra are rectangular while in the crane fly, ***Tipula paludosa***, they are crescent-shaped or rather like segments of an orange.

The shape of the crystal appears to be governed by the virus rather than by the host cell. This was first pointed out by Gershenson (1959, 1960) who isolated a strain of virus from ***Antheraea pernyi*** Guérin-Méneville which induced the formation of a hexagonal polyhedron instead of the more usual tetragon-tritetrahedral shape. Inoculation of healthy larvae with these hexagonal polyhedra produced a disease with polyhedra all of this shape; in other words the strain 'breeds true'. This is important because the shape of the polyhedra can be used as a 'marker' to pinpoint a virus strain and thus becomes a useful tool in the study of virus interference and mixed virus infections. The same phenomenon occurs in other inclusion-body diseases as will be seen later. It should perhaps be pointed out that abnormal polyhedra produced by heat or other artificial methods do not give rise to similar abnormal polyhedra when inoculated to susceptible larvae but only to the polyhedra characteristic of the source of inoculum.

Thin sections of polyhedra, viewed under the electron microscope, reveal the virus rods scattered apparently at random within the polyhedral crystal. They may occur singly, in groups or in bundles of ten or more, thus giving rise to the name 'bundle virus'. The polyhedra are fairly easily dissolved in weak sodium carbonate

and this releases the virus rods leaving behind an empty balloon-like structure which has been variously interpreted as an artifact or a membrane. The envelope or 'membrane' surrounding the nuclear polyhedra may be only a thin layer of hardened protein that is more resistant to dissolution than the interior polyhedral matrix protein. This envelope, however, is apparently permeable to the alkali at certain pH and molar conditions (Nordin and Maddox, 1971). In addition, the virus rod itself is contained in two membranes, an inner and an outer. These membranes have been studied on the electron microscope by Ponsen *et al.* (1964, 1965) in the nuclear polyhedroses of ***Malacosoma neustria, Barathra brassicae*** and ***Adoxophyes reticulata***. They find that the outer virus membrane appears to be composed of a central bimolecular leaflet of lipids bounded on either side by carbohydrates and proteins. The polyhedral and inner virus membranes however seem to consist of protein only. The inner membrane is closely attached to the virus rod, and so is the polyhedral membrane to the polyhedral protein. In the granuloses the outer portion of the crystal stains more deeply, simulating a membrane-like cover (see Fig. 3.2).

Examination of ultrathin sections of nuclear polyhedra at very high magnification under the electron microscope reveals a regular dot and line pattern. This is very well shown also in thin sections of another type of crystalline inclusion, the capsules of the granuloses (see Fig. 3.2). Bergold (1963a) has made a study of the crystalline lattice in the polyhedra of various species by means of X-rays and electron microscopy of very thin sections calculated to be only 100 Å thick and his conclusions are given here. The crystalline lattice has a very high degree of regularity without dislocations, the protein molecules being spheres with a diameter of about 90 Å (Hall, 1960) but this diameter may vary from 65–90 Å according to the insect species from which the polyhedra have been obtained. Between the rows of molecules angles of 90° and 120° could readily be observed. All the observed dot and line patterns can be explained with the aid of light and X-ray micrographs of molecule models arranged in a cubic system but cut at different angles.

The nuclear polyhedra are insoluble in water but dissolve readily in aqueous solutions of NaOH, KOH, H_2SO_4 and CH_3COOH, but the polyhedra from different nuclear polyhedroses differ greatly in their resistance to alkali treatment and this has to be taken into account when isolating the virus from them. With some viruses there is a critical point in the dissolution of the polyhedra beyond which the virus also is dissolved.

The most resistant polyhedra are those from ***Tipula paludosa***, the crane fly (Smith and Xeros, 1954a), and from the Australian pasture caterpillar ***Pterolocera amplicornis*** Walker (Day *et al.*, 1953).

As already mentioned, the polyhedra consist of protein; Bolle (1874, 1894) was the first to prove this and to show that they contained no lipid. The virus represents about 5 per cent of the whole crystal. Ribonucleic acid, RNA, and deoxyribonucleic acid, DNA, are present in the polyhedra of some species (Tarasevich, 1946; Faust, 1966; Faust and Estes, 1965; Estes and Ignoffo, 1965; Aizawa and Iida, 1963). Silicon has also been detected in some nuclear polyhedra (Estes and Faust, 1966; Faust and Adams, 1966).

The viruses of nuclear polyhedroses, as the name implies, multiply exclusively in the cell nuclei, mainly in the skin, blood cells, fat body, tracheae and occasionally the silk glands (Aruga *et al.*, 1963). There are exceptions, however, to these multiplication centres; in sawfly larvae the polyhedra occur only in the nuclei of the midgut cells (Balch and Bird, 1944; Bird and Whalen, 1953). Other examples of this type of infection are in larvae of a moth, ***Plusia chalcytes*** (Laudeho and Amargier,

1963), and occasionally in the silkworm, ***Bombyx mori*** (Watanabe ***et al.***, 1967).

The occurrence of nuclear polyhedra in midgut cells may lead to confusion with cytoplasmic polyhedroses in which the virus also attacks the cells of the gut but the fact that the latter is never in the cell nuclei should suffice to differentiate between the two. As a rule in the early stages of the disease the caterpillar becomes sluggish and refuses to eat. The first sign of the disease usually appears in the skin which may become oily as in the larvae of the butterflies ***Vanessa io*** and ***Aglais urticae*** or develop yellow patches as in the silkworm. As the disease progresses the skin becomes fragile and finally ruptures, liberating a milky fluid which is a suspension of many thousands of polyhedra. It was probably from this liquefaction of the body contents that the name 'caterpillar wilt' was derived.

Precocious development of adult characteristics in larvae (Prothetely) has been observed in lepidopterous larvae infected with NPV. This takes the form of antennae, mouthparts of sucking type, adult-type fore legs and partial fusing of the ocelli.

The precocious development of adult characteristics indicates an unnatural increase in the growth-promoting hormone ecdysone (Morris, 1970a).

Adult monstrosities have been observed arising from caterpillars infected with the ***Tipula*** iridescent virus (TIV) (Smith ***et al.***, 1961).

A characteristic of the nuclear polyhedroses is the tendency of affected caterpillars, in a late stage of the disease, to seek the highest point available, whether it be the lid of the cage or the top of a tree, and thence to hang head downwards. In forests in Germany where epizootics of nuclear disease are common in mass infestations of nun moth, ***Lymantria monacha*** Linn., and gypsy moth, ***Porthetria dispar*** Linn., caterpillars, the phenomenon is called 'Wipfelkrankheit' or 'tree-top disease'.

Lepidoptera

The nuclear polyhedrosis of the silkworm, *Bombyx mori* L.

The polyhedra As already stated, these vary in size and shape, although the prevailing shape is that of a dodecahedron. Ribonucleic acid (RNA) is present in the polyhedra (Tarasevich, 1946) as well as deoxyribonucleic acid (DNA) (Faulkner, 1962). The latter also reported the presence of as much RNA in the inclusion body as there was DNA in the virus particle. Himeno and Onodera (1969) have also isolated RNA from the nuclear polyhedra. Their results suggest that it is the messenger RNA for polyhedral formation and the RNA is included during the formation of the polyhedra.

The virus particle The DNA content of the virion is about 7.9 per cent (Bergold and Wellington, 1954).

As with the viruses of all nuclear polyhedroses, the particle is rod-shaped, measuring about 330 x 80 nm, inclusive of the inner and outer membranes. The surface of the particles is rough and knobbly and there is sometimes a terminal protrusion. Smith and Hills (1962a) treated the purified virus with weak alkali and examined the disintegrated virions under the electron microscope with negative staining. The first stage in the break-down is the peeling off of the outer membrane.

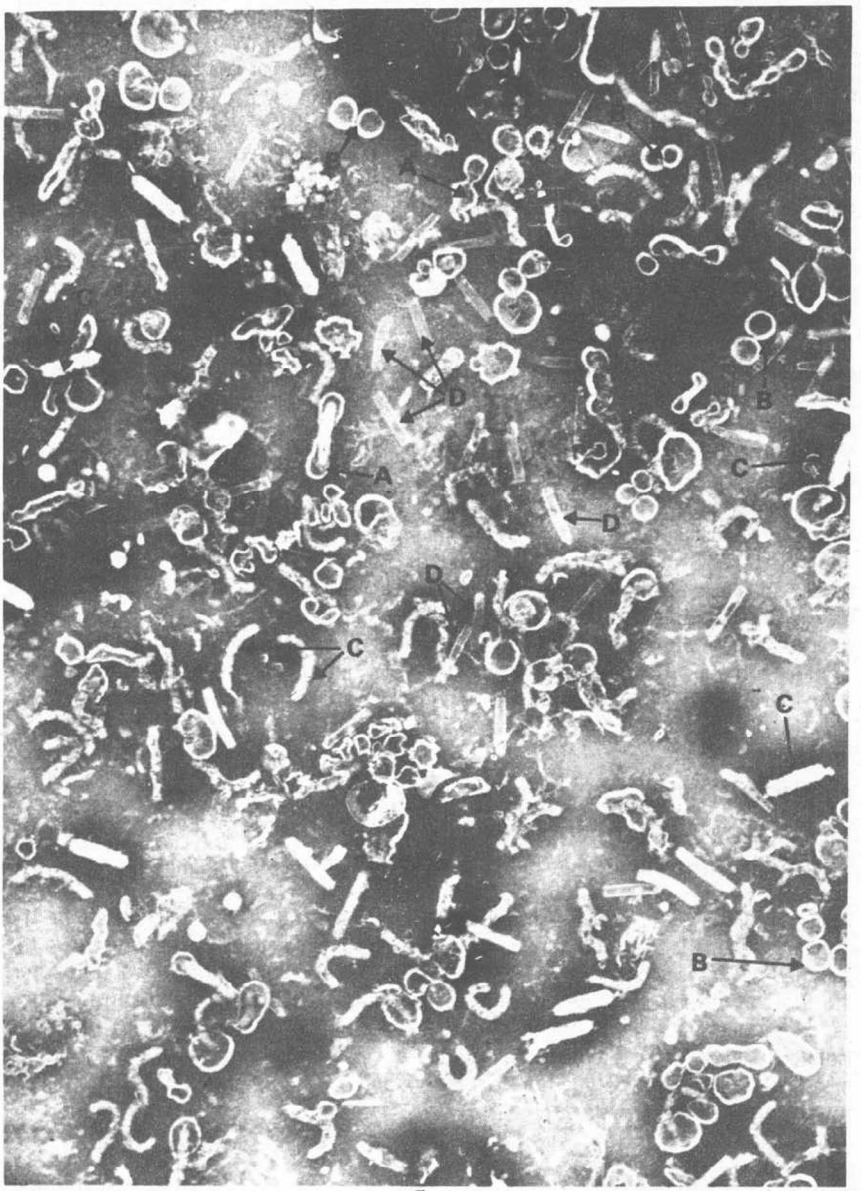

Fig. 1.1 The virus from the nuclear polyhedrosis of the silkworm treated with weak alkali. Note (A) the outer membrane breaking in the centre and folding backward; (B) the two spheres thus formed; (C) the contents of the intimate membrane which, at high magnification, appear to be helical (in some cases a terminal protrusion is visible); (D) the empty intimate membrane. x32,000. (K. M. Smith and G. J. Hills, 1967. Academic Press.)

This breaks in the centre and folds backwards, thus forming two spheres joined in the middle. These finally break apart and are thought to be the same as the spherical 'subunits' which Bergold said were discharged from the intimate membrane (Bergold, 1958). The intimate membrane is then exposed; in this instance it measures about 20 Å in thickness and has a slightly different structure at either end. At very high magnification the contents of the intimate membrane give the appearance of a widely spaced helix; these contents are discharged from either end of the intimate membrane and appear to uncoil as they flow out. This helix is considered to be in part deoxyribonucleic acid (DNA) (Fig. 1.1). A more recent study on similar lines has been done by Khosaka ***et al.*** (1971). They also broke down the virus rods with alkali and separated out the various constituent parts on a sucrose gradient. They found that the virus rods, without their membranes, were somewhat more elongated and narrower than the complete rod; 360 x 60 nm as compared with 330 x 80 nm. The empty inner membranes appeared to have cross striations on the surface and the same spheres resulting from the outer membranes as those shown by Smith and Hills were also demonstrated. The contents of the inner membrane, however, had disintegrated (Figs 1.2, 1.3). The amino acid contents of the polyhedra and the virus particle respectively are given in Tables 1.1 and 1.2 reproduced from Kawase (1964).

Disease caused For the first few days after infection the larvae show no symptoms other than sluggishness and lack of appetite. Later yellow patches may develop on

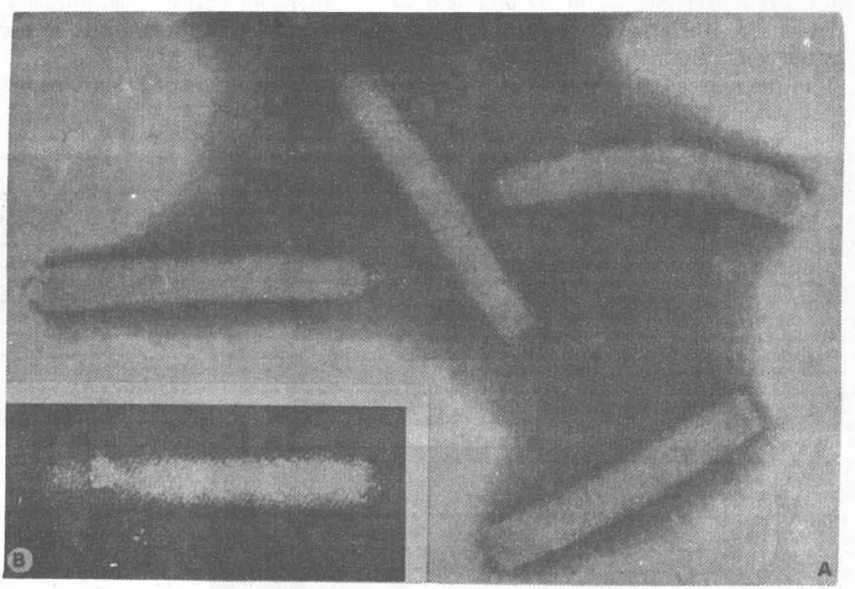

Fig. 1.2 (A) Slender rod-shaped particles of the nuclear polyhedrosis of the silkworm, about 360 nm long and 60 nm wide, somewhat elongated and distorted. x100,000. (B) Particle showing surface banding and internal substance x100,000. (Courtesy T. Khosaka, M. Himeno and K. Onodera, 1971.)

the skin from which the term 'silkworm jaundice' is derived. The skin then becomes very fragile and ruptures at a touch, releasing the milky haemolymph containing the polyhedra. The latter are mainly confined to the nuclei of the blood cells, fat body, tracheal matrix and epidermis. However, large polyhedra have been observed in the middle and posterior portions of the silk gland (Aruga ***et al.***, 1963). DNA synthesis has been studied in silkworm pupae after infection with NPV. Infection resulted in a marked change in the rate of DNA synthesis in the pupal bodies. The change was found to be due to the apparent DNA polymerase activity. The activity did not increase until 20 hours after infection, and then increased logarithmically from 24 and 48 hours. Sixty hours after infection the activity was twenty times that in normal pupae and then began to decrease (Onodera ***et al.***, 1968). The purified viral DNA is infectious and it is double-stranded (Onodera ***et al.***, 1965).

A study by radioautography of the silk-gland cell of a silkworm infected with NPV has revealed an alteration in the silk-protein synthesis. The results suggest that the synthetic activity of silk-protein in the silk-gland cell is greatly lowered by infection with the virus while, on the other hand, synthesis of polyhedron protein becomes active as the disease progresses (Watanabe and Kobayashi, 1969). Radioautography has also been used to study the patterns and changes of nuclei acid synthesis during the course of nuclear polyhedrosis. Tritiated thymidine and uridine were employed as nucleic-acid precursors. The results indicated that syntheses of DNA and RNA in the infected nuclei of the fat body increased progressively up to a point just prior to polyhedra development. Beyond this point there was a sudden break-down of DNA synthesis while the activity of RNA synthesis decreased gradually with the polyhedral growth. Some of the newly synthesized RNA in the diseased nuclei seemed to be adsorbed on to polyhedra during their formation (Watanabe, 1967a). Presumably this is not the RNA previously referred to as part of the composition of the polyhedra.

In the nuclear polyhedrosis of the midgut, the incorporation of 3H-uridine into

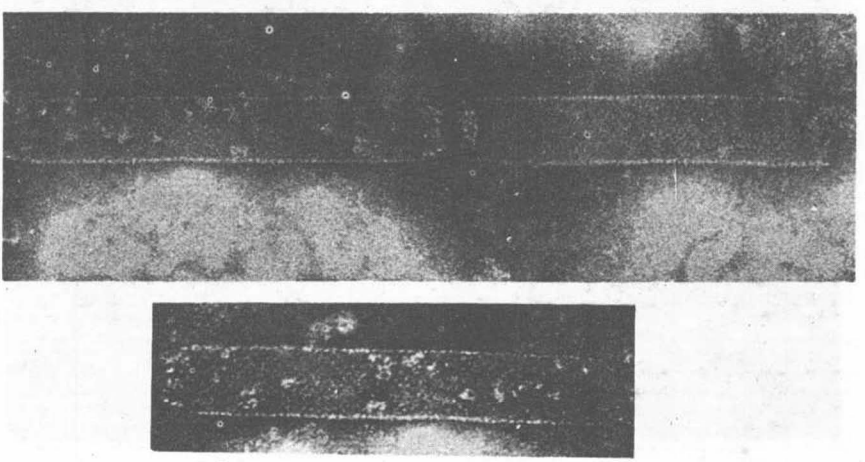

Fig. 1.3 Empty virus rods showing regular striations. x92,000. (Courtesy T. Khosaka, M. Himeno and K. Onodera, 1971.)

Table 1.1 Amino acid composition of polyhedron proteins in the silkworm[a, b, c]

	Hexahedral cytoplasmic inclusions	Icosahedral cytoplasmic inclusions	Nuclear polyhedra
Lysine	6.1	6.3	9.7
Histidine	2.4	2.2	2.2
Ammonia	3.5	4.2	3.6
Arginine	6.2	6.1	6.3
Aspartic acid	13.4	13.6	13.3
Threonine	2.5	3.0	4.3
Serine	7.9	7.6	4.0
Glutamic acid	11.6	12.4	14.3
Proline	3.3	3.2	5.4
Glycine	3.1	3.5	3.3
Alanine	5.1	5.5	3.8
Cystine	0.7	0.9	1.0
Valine	9.3	9.5	6.8
Methionine	1.5	1.3	2.0
Isoleucine	6.7	7.5	6.3
Leucine	7.8	8.6	8.9
Tyrosine	9.8	8.2	8.1
Phenylalanine	6.5	4.8	6.8
Alloisoleucine	0.1	0.1	0.1
Tryptophan[d]	2.3	2.2	2.8
Total	109.8	110.7	113.0
Total calculated as amino acid residue	91.5	91.6	94.7

a Data from Kawase (1964).
b Material: C122 x N124.
c Expressed a g amino acid per 16 g nitrogen.
d Determined by *p*-dimethylaminobenzaldehyde method.

both nuclear and cytoplasmic RNA was slightly larger than in the healthy control (Watanabe, 1967b).

By means of electrophoretic separation it has been shown that the haemolymph of diseased larvae contains less protein than that of normal larvae. This was most noticeable in the fat body which, besides being the centre of haemolymph protein synthesis, is also one of the most susceptible tissues to the NPV (Watanabe ***et al.***, 1968). The lipid content of the haemolymph in the diseased silkworm is higher than that of the normal, and the amount of phospholipid and cholesterol increases markedly after the release of polyhedra into the haemocoel (Komano ***et al.***, 1966).

The amount of peptides in the blood plasma of diseased larvae is higher than in the case of healthy larvae. This increase in peptides may be mainly due to the decomposition of proteins in the tissues (Kawase, 1966).

The amounts of phosphorus compounds were determined in larval haemolymph and in whole pupae during the course of virus infection.

Total P and acid soluble P/total P decreased at the later stage of infection, while lipid P and nucleic acid P increased at the time of the symptom appearance with larval haemolymph. Total P and nucleic acid P of the pupal haemolymph showed a similar result to that of the larval haemolymph (Iida and Aizawa, 1962).

Table 1.2 . Amino acid contents of silkworm viruses[a, b, c]

	Hexahedral cytoplasmic inclusions	Icosahedral cytoplasmic inclusions	Nuclear polyhedra
Lysine	5.8	5.7	5.4
Histidine	2.8	2.1	Trace[d]
Ammonia	4.0	4.6	4.0
Arginine	6.5	6.4	5.5
Aspartic acid	12.5	11.4	12.7
Threonine	4.4	4.4	6.2
Serine	6.9	6.9	6.1
Glutamic acid	10.6	10.4	6.2
Proline	4.7	4.8	7.1
Glycine	4.4	5.0	6.4
Alanine	5.6	6.0	7.4
Cystine	Trace	0	0
Valine	5.8	6.6	5.9
Methionine	2.0	1.0	1.5
Isoleucine	5.1	6.1	5.7
Leucine	7.5	8.2	9.2
Tyrosine	6.1,	4.3	4.7
Phenylalanine	5.4	6.0	6.2
Alloisoleucine	0	Trace	Trace
Grams of amino acid recovered per 100 g of virus	65.4	66.4	70.2

a Data from Kawase (1964).
b Material: Keno x Shunpaku.
c Expressed as percentage of total recovered amino acids.
d In this sample histidine could not be calculated because of interference by ammonia.

Purification of the virus In the nuclear polyhedrosis of the silkworm, as with other inclusion-body diseases, the first step in the isolation of the virus is the preparation of a pure suspension of the polyhedra. Since they are insoluble in water this is fairly straightforward. Larvae in a late stage of the disease can be cut up and suspended in water in a large flask. They can then be left for some days to allow the bodies to putrefy and disintegrate; the polyhedra will gradually settle to the bottom of the flask as a white layer. The next step is to decant the supernatant containing the larval debris and resuspend the polyhedra in water. Further purification by alternate high and low speed centrifugation yields a white preparation of polyhedra, comparatively free from impurities. The process can be speeded up by grinding the diseased larvae in a mortar and washing the resulting paste through cheesecloth in a filter funnel. A certain proportion of polyhedra is lost by this method, possibly by adsorption to the host tissues during the grinding-up process.

Further separation and purification of the polyhedra can be obtained by the use of fluorocarbon. They are suspended in a fluorocarbon–water mixture (1 : 1) and well shaken. The mixture is then centrifuged briefly and the supernatant which should still contain the polyhedra can be used or shaken again with fluorocarbon (Bergold, 1959).

The next step in the isolation of the virus rods is to extract them undamaged from the polyhedra. This is best accomplished by the careful dissolution of the polyhedra with weak sodium carbonate. The concentration of the sodium