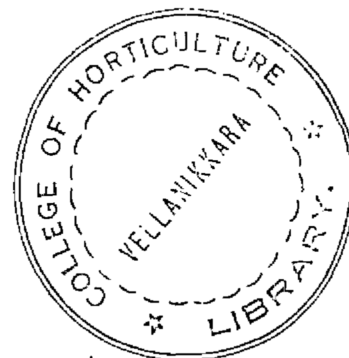
INVESTIGATIONS ON NUCLEAR POLYHEDROSIS OF BLACK HAIRY CATERPILLAR, *PERICALLIA RICINI* FABRICIUS (ARCTIIDAE : LEPIDOPTERA)



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THESIS

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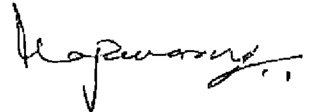
(P.A. Rajan Asari) 

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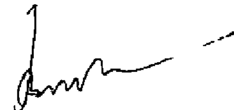
4. 

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C E R T I F I C A T E

Certified that this thesis is a record of research work done independently by Sri.K.P. Vasudevan Nair, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

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Pathogenic microorganisms like viruses, bacteria, fungi, nematodes and protozoa are well known weapons in the armoury of economic entomologists for subjugating insect pests of crops. Many of these microbial agents have been tested for their ability to control pest species and for their suitability for development as microbial insecticides. At least ten insect pathogens are presently available as microbial insecticides and over fifty genera of insect pathogens are recommended as additional candidates for mass production (Ignoffo, 1967).

The rapidly expanding field of microbial control is today mainly concerned with the exploitation of a wide range of virus diseases of insects. Much work has been done in other countries like U.S.A., Canada, U.K., France and U.S.S.R. in this field and now there are over thirty successful reported instances in which insect viruses have been used for the control of crop pests. The most outstanding successes were achieved with the use of nuclear polyhedrosis viruses (Balch and Bird, 1944; Thompson and Steinhaus, 1950; Bird, 1953 a; Clark and Thompson, 1954; Vago and Cayroll, 1955; Hall, 1957;

Ossowski, 1957; Abul Nasr, 1959; Smirnoff, 1961; Ignoffo et al. 1965). In U.S.A. commercial preparations of five insect viruses, all nuclear polyhedroses, have been made available by industry and the preparation containing nuclear polyhedrosis virus of Heliothis has been recommended for field use as a microbial insecticide (Ignoffo, 1968).

The science of insect pathology, especially the study of virus diseases is still in its infancy in India. A few cases of virus infection in insects have been reported and these include the nuclear polyhedrosis on Heliothis armigera (Patel et al. 1968; Jacob and Subramaniam, 1972 a), Prodenia litura (Ramakrishnan and Tiwari, 1969), Amsacta albistriga and Spodoptera litura (Jacob and Subramaniam, 1972 a), Antheraea mylitta (Pattar and Mathad, 1972), Diacrisia obliqua (Jacob and Thomas, 1972) and Spodoptera mauritia (Jacob et al. 1973) and a cytoplasmic polyhedrosis on Heliothis armigera (Rabindra and Subramaniam, 1973). Granuloses have been reported from Cnaphalocrocis medinalis (Jacob et al. 1971), Diacrisia obliqua and Spodoptera litura (Battu et al. 1971) and Pericallia ricini (Jacob et al. 1972). A pox like virus has been reported to occur in Amsacta moorei (Roberts and

Granados, 1968; Mathur, 1971). Detailed investigations on the host-pathogen relationships have been conducted on the nuclear polyhedrosis of P. litura (Pawar and Ramakrishnan, 1971 a, b), S. litura (Jacob, 1972) and S. mauritia (Lathika, 1973).

The black hairy caterpillar, Pericallia ricini Fabricius is a polyphagous pest feeding on a variety of crop plants like cotton, castor, banana, cucurbits, field beans, gingelly etc. Occurrence of a nuclear polyhedrosis in this insect was first recorded by Jacob et al. (1972). Nut no information is available on the potentiality of this pathogen for microbial control. The present investigations were hence taken up to gather much needed basic information on various aspects such as symptomatology, larval susceptibility, effect of virus infection on the moulting of larvae, changes in the number of circulating haemocytes in the infected larvae, nature of causative agent, effect of physico-chemical factors on the virus and the cross infectivity of the virus to other species of Lepidoptera.

REVIEW OF LITERATURE

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Insect viruses are broadly classified into inclusion viruses and non-inclusion viruses. Inclusion viruses are characterised by the presence of a unique proteinaceous crystalline structure called an inclusion body. The infective virus particles or virions are occluded in the protein matrix of these inclusions. Ninetyfive per cent of the arthropod viruses so far described are inclusion viruses and the non-inclusion viruses comprise the remaining five per cent.

Diseases characterised by the presence of inclusion bodies with sharp distinct sides forming regular or irregular polyhedra are called polyhedroses. Eighty per cent of the insect viruses are polyhedroses. When the inclusion bodies are formed in the nuclei of the infected cells the diseases are called nuclear polyhedroses. Cytoplasmic polyhedroses are diseases in which the inclusion bodies are formed in the cytoplasm of the infected cells. A brief review of literature on nuclear polyhedroses with special reference to the present investigations is presented in the following pages.

Early History

Much of the early work on what are now known as virus diseases were carried out an 'Jaundice' of silkworm (*Bombyx mori*). Earliest description of this disease was by Nysten (1808). Cornalia (1856) and Maestri (1856) independently reported for the first time the presence of polyhedra in the tissues of the jaundice affected silk-worms. But the exact relationship of these polyhedra with the cause of the disease was established only many years later. Komarek and Briendl (1924) first suggested that the causative agent of the disease might be contained within the polyhedra. This was later confirmed by Bergold (1947) who demonstrated the presence of the virus in the polyhedra by electron microscopy. Rapid progress was made in the study of insect viruses during the years followed and a large number of inclusion and non-inclusion viruses were described.

Distribution and Host range

Ignoffo (1968) estimated that nuclear polyhedroses represented about 44 per cent of all the described insect viruses. Majority of the nuclear polyhedroses occur in the larvae of Lepidoptera while a limited number of this

disease has also been reported from few hymenopterous and dipterous insects (Smith, 1967).

Symptomatology

The symptoms due to nuclear polyhedrosis virus infection vary in different insects. The common symptoms exhibited by lepidopterous larvae infected with nuclear polyhedrosis as reviewed by Aizawa (1963) and Smith (1967) may be summarised as follows: The first symptoms appear in the skin and in some larvae the skin takes an oily appearance. Colour changes or mottled appearance of the cuticle are also common. As the disease advances the larvae become lethargic, lose their appetite and stop feeding. The cuticle becomes exceedingly fragile and ruptures finally liberating the body contents which are liquefied by this time. In the late stages of the disease the larvae show a tendency to seek the highest point available and thence to hang head downwards. The incubation period of the disease may vary from 3 days to 3 weeks.

Tanada (1954) observed that larvae of *Pieris rapae* became lighter in colour and appeared mottled as a result of nuclear polyhedrosis infection. Bloated appearance of infected larvae had been observed in *Pieris rapae*

(Tanada, 1954), Trichoplusia ni (Drake and McEwen, 1959) and in Spodoptera litura (Jacob, 1972). Death of larvae infected with nuclear polyhedrosis virus without showing any typical symptom was observed in Heliothis peltigera by Harpaz and Zlotskin (1965). Similar observations were made by Jacob (1972) in the larvae of Heliothis armigera.

Vago (1950, 1956) observed that nuclear polyhedrosis virus caused moulting disturbances. Morris (1970) reported precocius development of antennae, mouthparts, adult type forelegs and partial fusing of oscelli in the larvae of Orgya psuedatsugata and Lambdina fiscellaria somniaria when infected with nuclear polyhedrosis in the fourth instar. Jacob and Subramaniam (1974) found that nuclear polyhedrosis infection inhibited moulting in Spodoptera litura in later stages of the disease. Rabindra and Subramaniam (1974) also made similar observations in the larvae of Heliothis armigera.

Size and shape of Polyhedra

The size and shape of nuclear polyhedra vary considerably both in different insects and also in different tissues of the same insect. According to Gerhenson (1959, 1960) it was the virus that controlled the shape of the

polyhedra rather than the host cell. The polyhedra from Tipula paludosa were usually crescent shaped (Smith and Xeros, 1954) while in scarlet tiger moth, Panaxia dominula the polyhedra were rectangular in shape (Smith, 1955) Bergold (1963) pointed out that in the silkworm, Bombyx mori the prevailing type of polyhedra were dodecahedra, whereas those of Lymantria monacha consisted mostly of tetrahedra.

The diameter of polyhedra has been reported to vary generally from 0.5 to 15 μ according to the species (Smith 1967). Tanada (1960) observed larger polyhedra in Spodoptera mauritia which varied in diameter from 1.07 to 3.22 μ. In the case of a nuclear polyhedrosis of Barathra brassicae, the diameter varied from 0.8 to 2.7 μ.(Ponson and de Jong, 1964) Hunter and Hall (1968) found that the polyhedra of Spodoptera exigua varied in diameter between 1 and 6 μ with an average of 2.05 μ. Lathika and Jacob (1974 b) reported that polyhedra from Spodoptera mauritia had an average diameter of 1.13 μ with a range from 0.44 to 1.76 μ.

Physico-chemical properties of polyhedra

Verson (1872) first suggested the crystalline nature of polyhedra and this was confirmed later in

preliminary X' ray investigations (Bergold and Brill, 1942) and electron microscopy (Morgan *et al*. 1955).

Bolle (1894) was the first to investigate the properties of polyhedra and he found that they were insoluble in cold and hot water, alcohol, ether, chloroform and acetone and that they were heavier than water. Bergold (1958) found that *Bombyx mori* polyhedra had a density of 1.268. Bergold (1959) reported that polyhedra were resistant to natural putrification but dissolved in aqueous solutions of NaOH, KOH, NH_3, H_2SO_4 and CH_3COOH. Benz (1963) considered that the resistance to bacterial putrification could be partly due to the outer membrane of polyhedra.

The polyhedra from different hosts have been found to differ greatly in their resistance to alkali treatment. Day *et al*. (1953) observed that polyhedra from Australian pasture caterpillar *Pterolocera amplicornis* required 60 minutes of exposure to 4 per cent sodium carbonate at a temperature of 56°C to dissolve them completely. Ignoffo and Dutky (1963) studied the dissolution of polyhedra with sodium hypochlorite and found that it was related to the concentration of hypochlorite and the length of exposure.

Brown and Swaine (1965) found that polyhedra of Spodoptera exempta dissolved quickly in 0.02 per cent NaOH while 2 per cent Na_2CO_3 was ineffective in dissolving them even after 30 minutes. The polyhedra of Prodenia litura were completely dissolved by 0.2 per cent KOH or NaOH in one minute while 5 per cent Na_2CO_3 solution required 30 minutes to produce the same result (Pawar and Ramakrishnan, 1971 b). Lathika and Jacob (1974 b) reported that polyhedra from Spodoptera mauritia dissolved completely in 0.2 per cent KOH or NaOH and 10 per cent Na_2CO_3 in less than 2 minutes and 0.1 per cent KOH or NaOH and 5 per cent Na_2CO_3 produced such an effect in 3 and 10 minutes respectively. Jacob and Thomas (1974) found that polyhedra from Diacrisia obliqua dissolved in 0.1 per cent KOH or NaOH in one minute but 5 per cent and 10 per cent Na_2CO_3 required 30 and 55 minutes respectively to dissolve them completely.

Smith and Xeros (1954) reported a peculiar nature of polyhedra from Tipula paludosa. They were insoluble in weak alkali or acid but in 1N NaOH they elongated to several times their normal length and returned to their normal size and shape when put in water.

Tarasevich (1945) reported that the Bombyx mori polyhedra had an isoelectric point of pH 5.2. According to Bergold and Schramm (1942) the polyhedral proteins were completely insoluble at their isoelectric points.

Chemical composition of polyhedra

Bolle (1874, 1894) was the first to analyse the nuclear polyhedra and found them to consist of protein and contain no lipids. Bergold (1947) reported that the polyhedral protein constituted about 95 per cent of the total weight and the virus particle about 5 per cent. Morgan et al. (1956) found that polyhedra of Porthetria dispar and B. mori contained 95 per cent protein.

Analyses of seven different polyhedrosis viruses and their inclusion bodies by Wellington (1951, 1954) showed that all had similar pattern of aminoacid composition which differed from the pattern of surrounding inclusion body proteins. She found a strikingly greater content of arginine and serine in the virus than in the polyhedra but the latter had more lysine and tyrosine. However Kawase (1964) found more argenine in the inclusion body than in the virus.

Faulkner (1962) reported that the polyhedra of B. mori contained RNA. According to Aizawa and Iida (1963) both DNA and RNA were always present in the polyhedra of B. mori. The RNA content was found to vary with the silkworm strain. Estes and Ignoffo (1965) studied the nucleic acid composition of polyhedra from Heliothis zea and found them to contain DNA at 6.81 ± 0.14 µg/mg and RNA at 2.02 ± 0.03 µg/mg polyhedra. Faust and Estes (1965) found that the polyhedra of Trichoplusia ni contained DNA and RNA at 12.02 ± 0.06 and 8.7 ± 0.09 µg/mg polyhedra respectively.

Studies by Holoway and Bergold (1953, 1955) revealed the presence of iron and magnesium in the inclusion bodies of B. mori. Estes and Faust (1966) observed a silicon content of 0.12 per cent in the polyhedra of H. zea and they suggested that the dissolution of the polyhedral protein in the insect gut was primarily dependent on the solubilization of the silicates present in the polyhedra.

Virus particle

The morphological characters of the nuclear polyhedrosis virus particle as described by Smith (1967) may be summarised as given below. They are always rod shaped and are enclosed in an outer developmental membrane and

an inner intimate membrane. More than one virus particle may be present within a developmental membrane but an intimate membrane will contain only one viral unit.

Approximately 10 to 100 virions are embeded singly or in bundles within each polyhedra and each rod average 400 x 80 mμ in size (Ignoffo, 1968). According to Morgan (1956) in Lepidoptera the virus rods were arranged haphazardly within the polyhedra. In *Tipula paludosa* Smith and Xeros (1954) observed that the virus rods were arranged in lines. The virus rods occured singly in the polyhedra of *Tipula paludosa* (Smith and Xeros, 1954), in *Heliothis armigera* (Bergold and Ripper, 1957; Jacob and Subramaniam, 1972 a) and in *H. zea* (Gregory *et al*. 1969). Bergold (1963) recorded upto 19 rods in one bundle in the polyhedra of *Lymantria monacha*.

Chemistry of the virus

Briendl and Jiroveo (1936) made the first suggestion that DNA was associated with nuclear polyhedrosis viruses. Gratia *et al*.(1945) made the first quantitative examination of whole polyhedra of *B. mori* and found them to contain 0.48 per cent DNA and no RNA. An analysis of highly purified virus particles of B. mori by Bergold and

Wellington (1954) revealed about 7.9 per cent DNA and 0.915 per cent phosphorus. Kreig (1956) found that the nuclear polyhedrosis virus of <u>Aporia crategi</u> (Linn.) contained 9 per cent DNA but no RNA. Morris (1962 b) reported that the nuclear polyhedrosis virus of western oak looper, <u>Lambdina fiscellaria somniaria</u> contained 7.9 per cent DNA. According to Ignoffo (1968) all nuclear polyhedrosis viruses so far described were DNA viruses.

<u>Site of virus multiplication</u>

As the name implies the virus multiplies in the cell nucleus. The tissues usually infected are the epidermis fat body, blood cells and tracheae. Several other tissues such as silkglands, muscle cells, nerve sheath, brain, ganglia, gonads, Malpighian tubules, connective tissues surrounding the midgut, epithelia of the fore and hindgut and imaginal wing buds have also been reported as susceptible to the virus (Tanada, 1960; Benz, 1963; Aruga <u>et al</u>. 1963 a; Stairs, 1965 b; Adams <u>et al</u>. 1968; Mathad <u>et al</u>., 1968; Hamm, 1968; Vail and Hall 1969 a; Jacob and Subramaniam, 1973).

Multiplication of nuclear polyhedrosis virus in the endodermal cells is rather unusual. Balch and Bird (1944)

and Bird and Whalen (1953) observed that in saw flies *Gilpinia heroyneae* and *Neodiprion sertifer* the virus multiplied in the nuclei of the epithetial cells of the midgut. Laudeho and Amargier (1965) found virus multiplication in the midgut cells of a lepidopterous larvae *Plusia chalcytes* (Esp.).

Larval age and susceptibility

Differential susceptibility of larvae of different ages to virus infections have been reported in several cases. Bergold (1943) found that the larvae of *B. mori*, *Porthetria dispar* and *Lymantria monaoha* did not die when infected with the virus in their more advanced stages of growth. According to Smith *et al*. (1953) *Sphinx ligustri* larvae were most susceptible during its early larval stage. Bird (1953 b) and Bird and Whalen (1953) observed that prepupal stages of the sawflies *Diprion hercyneae* and *Neodiprion sertifer* were immune to infection of polyhedrosis and suggested that the immunity was associated with the 'embryonic' cells lining the midgut during the prepupal stage. Clark and Thompson (1954) reported that larger doses of the virus were required to kill older larvae of forest tent caterpillar *Malacosoma disstria*.

Tanada (1956) found that the resistance of the armyworm, Pseudaletia unipuncta increased directly with the age of the larvae. Tanada (1960) reported that the last instar larvae of lawn armyworm, Spodoptera mauritia were fairly resistant to nuclear polyhedrosis virus. Decrease in susceptibility to polyhedrosis virus by older larvae had also been observed in Lambdina fiscellaria somniaria (Morris, 1962 a), M. disstria (Stairs, 1965 a), P.dispar (Doane, 1967), S. litura (Jacob and Subramaniam, 1972 b) and in S. mauritia (Lathika and Jacob, 1974 b). The increase in resistance associated with the growth of larvae has been regarded by some authors as " maturation immunity". Ignoffo (1966 a) attributed it partly to the normal increase in body weight which may serve to dilute a constant virus dose. Allen and Ignoffo (1969) found that the LD_{50} nuclear polyhedrosis virus for 8 day old larvae of H. zea was 250 times that of a 3 day old larvae in the laboratory.

There are few reports of older larvae being more susceptible to virus infections. Glasser (1928) found that all larval instars of B. mori were susceptible to poly-hedrosis virus, but older larvae were more easily infected than the young. Kreig (1955) did not detect any differen-tial resistance when Neodiprion sertifer larvae were fed with high virus doses.

Cross infectivity

Steinhaus (1953) found that alfalfa caterpillar, Colias, philodice eurytheme was susceptible to a nuclear polyhedrosis virus from the larvae of the South American species Colias lesbia. Tanada (1954) reported that the polyhedrosis virus of Pieris rapae appeared to be identical with the virus of Colias philodice eurytheme since these two viruses were naturally cross transmissible to both species. Clark and Thompson (1954) successfully transmitted the virus from Malacosoma californicum to M.fragile. Smirnoff (1963) reported a case of adaptation of a nuclear polyhedrosis virus of Trichiocampus viminalis to the larvae of T. irregularis by successive passage in the host. Ignoffo (1965) reported that six species of Heliothis were all susceptible to one isolate of nuclear polyhedrosis virus and the virus isolated from H. peltigera was cross transmissible to three of the above species.

Smirnoff (1962 b) reported a case of intergeneric transmission of a nuclear polyhedrosis virus of Eranis tiliaria (Geometridae) to two other species of Geometridae viz. Alsophila pometria and Phigalia telia. Morris (1964) observed that the virus from Caripeta devastata (Geometridae) could be transmitted to the larvae of

Lambdina fiscellaria somniaria and *Lambdina fiscellaria lugubrosa* (Geometridae).

Cross transmission is not always confined to related groups in the same families and genera. Aizawa (1962) found that larvae of *Galleria mellonella* (Galleridae) were susceptible to a nuclear polyhedrosis virus from the silkworm, *B. mori* (Bombycidae). Larvae of *Hemerobius* sp.(Neuroptera) were reported susceptible to a nuclear polyhedrosis virus of *Porthetria dispar* (Lepidoptera) (Smith *et al*. 1959; Sidor, 1960).

Pupal and Adult infections

Upto recent years the general opinion had been that lepidopterous adults were immune to virus infection, eventhough they might transmit the virus to their offspring in an active or latent state. However, Aizawa (1963) obtained infected adults of *B. mori* by inoculating the virus in the late pupal stage. Martignoni (1964) succeeded in infecting adult tissues of *Peridroma saucia* by inoculating nuclear polyhedrosis virus into adult tissues. Stairs (1965 b) observed pupal mortality when the larvae of *G.mellonella* were fed with the virus 3 to 5 days before pupation. Vail and Hall (1969 b) found that cabbage looper, *Trichoplusia ni* pupae were susceptible to nuclear polyhedrosis when the virus was injected into the haemocoel.

Haemocyte changes in virus infected larvae

Qualitative as well as quantitative changes have been reported to occur in the blood due to infection by nulcear polyhedrosis viruses. Shapiro (1967) observed a decrease in the number of haemocytes in the nuclear polyhedrosis infected larvae of wax moth, Galleria mellonella and the decrease was significant at or after 10 days. Zelinskava (1968) found that at an early stage of polyhedrosis of Porthetria dispar proleucocytes, macroleucocytes and active phagocytes appeared. Shapiro et al.(1969) recorded a drastic reduction of haemocytes in H. zea larvae exposed to a high dose of the nuclear polyhedrosis virus for 3 days but not in healthy larvae or in larvae exposed to a low dosage. Ramakrishnan and Tiwari (1972) observed that a gradual decrease in the blood cells of P. litura infected with a nulcear polyhedrosis virus. They also found that both plasmatocytes and granular cells were infected and the spindle cells (plasmatocytes) were found to be greatly reduced in diseased larvae. Jacob (1972) reported a significant increase in the number of haemocytes in the virus infected larvae of S. litura 24 hours after infection compared to the healthy larvae. However, at all subsequent intervals he observed a significantly lower number of haemocytes in the diseased larvae. In the

larvae of S. maurita Lathika and Jacob (1974 c) found that number of circulating haemocytes steadily decreased from 48 hours after infection with nuclear polyhedrosis. Rabindra and Subramaniam (1974) observed a decrease in THC of virus infected larvae of H.armigera from the third day of infection.

Generation to generation transmission

The transmission of virus from parents to offspring is known to occur in many Lepidoptera. It may take place by the virus that may be contained within the eggs (transovarial) or on the exterior of the eggs (transovum). Transovarial transmission was first suggested as occurring in B. mori by Conte (1907) and Bolle (1908). Sager (1960) observed virus like bodies in the eggs of seven species of Lepidoptera. Bird (1961) reported transovum transmission of nuclear polyhedrosis in 3 species of sawflies D.hercyniae, N. sertifer and N.leconte. Smirnoff (1961, 1962 a) observed virus transmission from adult to larvae through egg in sawflies N.swainei, Trichiocampus irregularis and T. viminalis. Martignoni and Milstead (1962) demonstrated that adults of Colias philodice eurytheme could transmit the virus to eggs and/or young larvae when the ovipositor was artificially contaminated. Elmore and Howland (1964)

obtained similar results with adult cabbage loopers. Harpaz and Benshaked (1964) found that nuclear polyhedrosis virus of Prodenia litura was transmitted through egg and that it involved a complicated genetio mechanism. Pawar and Ramakrishnan (1971 a) also made similar observations with the nuclear polyhedrosis virus of Prodenia litura. Vail quoted by Ignoffo (1968) detected virus inclusions associated with the ovaries of virus injected cabbage looper pupae. Doane (1969) demonstrated that transovum transmission of the virus in gypsy moth, P. dispar took place by surface contamination of eggs.

Mixed infections and synergism

Paillot (1936) observed mixed infection of a nuclear polyhedrosis and a granulosis virus in the larvae of Agrotis segetum. Tanada (1954) observed mixed infection of nuclear polyhedrosis and granulosis in Pieris rapae. Similar observations were made by Steinhaus (1957) in the larvae of Nephelodes emmedonia, Bird (1959) in Choristoneura fumiferana, Wittig (1959) in C. murinana, Paschke and Hamm (1962) in Trichoplusia ni, Steinhaus and Marsh (1962) in Spodoptera fugiperda and Jacob et al.(1972) in Pericallia ricini. Double infection of B. mori with both nuclear polyhedrosis and cytoplasmic polyhedrosis had been reported

by Smith (1967). Mixed infection of a nuclear polyhedrosis and microsporiosis was reported by Tanada and Reiner (1962 a) in the larvae of *Pseudaletia unipuncta*.

The occurrence of two viruses or virus and another pathogen in the same insect sometimes has a synergistic effect. Tanada (1959 a,b) reported that the granulosis virus had a synergistic effect to nuclear polyhedrosis virus in *P.unipuncta*. Steinhaus (1951) reported that a mixture of *Bacillus thuringiensis* and nuclear polyhedrosis was more efficient than virus alone against the larvae of *Colias philodice eurytheme.* Stelzer (1965) also found a similar mixture to be effective against great basin tent caterpillar, *Malacosoma fragile*. Bird (1969) could not find any evidence of synergism between the viruses of spruce budworm.

Aruga *et al*. (1961) observed a case of interference between strains of nuclear polyhedrosis virus in *Hypantria cunea.* Similar observations had been made by Aruga *et al*. (1963 b) in silkworm larvae between a strain of silkworm cytoplasmic polyhedrosis virus and a virus of the pine caterpillar, *Dendrolimus spectabilis*. Tanada and Chang (1964) observed that in the alfalfa caterpillar its

virus interfered with the infection by silkworm virus.

Environmental persistence

According to Smith (1967) inclusion viruses could persist longer than non-inclusion viruses because the proteinaceous crystals protected the virus from the effects of unfavourable environment. Ignoffo (1968) suggested that environmental factors such as light, temperature, rainfall, free water, pH, and plant interaction might deactivate field applied viruses. But normal range of physical components of the environment was not found to be destructive to most viruses.

Field applied viruses have been reported to persist in the soil for several years. Steinhaus and Thompson (1949) observed that alfalfa caterpillar exposed to soils from alfalfa fields developed typical symptoms of nuclear polyhedrosis. The soil inoculated with cabbage looper nuclear polyhedrosis retained infectivity upto two years and was detected on cabbage foliage after heavy rains (Jaques, 1964). Nuclear polyhedrosis virus and granulosis virus of T. ni were detected in soil samples collected from crucifer fields of southern ontario though no artificial introduction of virus was made in these fields (Jaques and Hercourt, 1971).

Laboratory tests by Ignoffo (1968) indicated that normal field temperatures (10-30°C) should not adversely inhibit viral activity or viral stability in diseased caterpillars. Continual exposure at higher field temperatures was found to affect viral stability and inhibit viral multiplication (Bird, 1955; Thompson, 1959; Ignoffo, 1966 b). The thermal inactivation of inclusions and enclosed virions was not different from the thermal inactivation of most proteins under laboratory testing (Bergold, 1953; Aizawa, 1953). Steurmer and Bullock (1968) found that the nuclear polyhedrosis virus of *Heliothis* was still infective when heated at 93.3°C for 30 minutes and was completely inactivated at 93.3°C in one hour. The thermal inactivation point of the nuclear polyhedrosis virus of *Prodenia litura* was found to be between 90 and 95°C when heated for 10 minutes (Pawar and Ramakrishnan, 1971 b). Morris (1971) reported that heating the nuclear polyhedrosis virus of *Lambdina fiscellaria somniaria* at 45°C for 200 hours did not affect the final percentage of mortality. Lathika and Jacob (1974 a) found that the thermal inactivation point of the nuclear polyhedrosis virus of *S. mauritia* to be between 90 and 95°C.

Ultraviolet light was reported to deactivate viruses under laboratory conditions (Watnabe 1951; Aizawa, 1955). According to Hirt *et al*. (1960) and Turner and Kaplan (1965), sunlight-uv, although not directly responsible, might indirectly activate or catalyse reactions which resulted in viral instability and loss of infectivity. Bullock (1967) observed that NPV of *Heliothis* applied to cotton foliage lost most of its viral activity after one day and that only slight activity persisted on the second day and suggested uv light to be a reason for this loss. However, studies by Morris (1971) suggested that ultra violet radiation of predominently 3600 A° wave length was only slightly viricidal and that gamma radiation of virus caused no substantial reduction in pathogenicity of the virus. But exposure to sunlight beyond 5 hours decreased pathogenicity of the nuclear polyhedrosis virus of *Lambdina fiscellaria somniaria*. The nuclear polyhedrosis virus of *Spodoptera mauritia* retained its infectivity even after 48 hours exposure to sunlight but was almost ineffective after 96 hours (Lathika and Jacob, 1974 a).

Compatibility with agricultural chemicals

Most chemical insecticides and insecticidal adjuvant have been found to be compatible with arthropod viruses.

Clark and Reiner (1956) found that Aracel-'c', Methocel, Span 80, Triton X-100, Triton B-1956 and diesel oil were compatible with the nuclear polyhedrosis virus of Colias philodice eurytheme. Out of 28 insecticides and adjuvants tested by Smirnoff (1961) only turpentine was found to be incompatible with the nuclear polyhedrosis virus of N. swainei. Many oils, insecticides and surfacants were compatible with the nuclear polyhedrosis virus of Heliothis (Tanada and Reiner, 1962 b; Wolfenbarger, 1964, 1965; Ignoffo et al. 1965). Similar results of compatibility were obtained for the nuclear polyhedrosis virus of cabbage looper McEwen and Hervey, 1958, 1959; Genung, 1960; Elmore, 1961; Hofmaster and Ditman, 1961; Getzin, 1962; Wolfenbarger, 1964; Ignoffo, 1964). Wolfenbarger (1965) found that oil and endrin when added to a virus-water solution gave better results than a threefold increase in virus concentration for control of cabbage looper. Ignoffo and Montaya (1966) tested six insecticides and six adjuvants (endrin, DDT, methyl parathion, toxaphene, toxaphene-DDT, carbaryl, triton B-1956, triton X-152, triton X-172, multifilm buffer-X xylene and carbonated water) for compatibility with nuclear polyhedrosis of Heliothis spp. of which only methyl parathion

adversely affected the infectivity of the virus. In a two year study of virus-chemical insecticide combination for the control of spruce budworm, *Choristoneura fumiferana* the application of NPV + fenitrothion combination was found to be highly effective (Morris and Armstrong, 1974).

MATERIALS AND METHODS

MATERIALS AND METHODS

Mass rearing of caterpillars of *Pericallia ricini*

Pneumatic glass troughs (12" x 5") glass battery jars (8" x 4"), specimen tubes, (3" x 1", 6" x 1"), plastic containers (150 ml) and hurricane chimneys were used for these rearings. The larger glass wares and chimneys were sterilized by keeping them in 0.5 per cent sodium hypochlorite solution (Wittig, 1963) for one day. They were then washed in running tap water and air dried. Smaller glass jars and tubes were sterilized in a hot air oven at 180°C for 3 hours.

The original culture was started from a single egg mass collected from the field. The eggs (One day old) were surface sterilized by immersing them in 10 per cent formalin for 90 minutes (Thompson and Steinhaus, 1950). They were then washed several times in distilled water and the moisture removed by air drying. The sterilized eggs were kept in clean sterilized petridishes for hatching.

On the day previous to hatching the eggs attained a bluish tinge when they were transferred to fresh castor leaves (*Ricinus communis* L.) kept in glass battery jars. The leaves were kept turgid by keeping the tips of petioles dipped in water contained in specimen tubes.

The jars were covered with muslin cloth. The leaves generally remained turgid for 2 to 3 days in this way. Larvae were transferred to fresh leaves after 3 days and reared like this untill the 5th day. Thereafter the larvae were transferred to glass troughs in batches of 20 to 25 per trough. A one inch layer of clean and sterilized (autoclaved) sand was provided at the bottom of the troughs. Fresh castor leaves were provided every day. The troughs were covered with muslin cloth. Larvae showing signs of bacterial or other infections were removed immediately. The larvae pupated on the leaves, sides of the trough and the cloth covering.

The adults on emergence were enclosed in battery jars or chimneys in batches of 3 or 4 pairs. Cotton swabs dipped in 10 per cent honey solution were pasted on the sides of the chimneys as food. The egg laying started within few hours of emergence and continued for 3 to 4 days. Fresh leaves were given every day for egg laying. Bits of castor leaves containing the egg masses were cut out and sterilized as described earlier.

Preparation and storage of primary inoculum

The primary inoculum was obtained from an infected laboratory culture in the Division of Entomology, College of Agriculture, Vellayani in 1973. It was multiplied by

feeding contaminated castor leaves to early instar larvae of P. ricini. The polyhedra were collected as described by Smith (1967). The diseased cadavers were stored in distilled water in large conical flasks and allowed to decay at room temperature for several weeks. The polyhedra which settled as a thin white layer at the bottom were collected and purified by filtration and differential centrifugation. The polyhedra were also extracted by maceration of dead larvae in a warring blender and further purification by filtration and centrifugation. The purified polyhedra were then suspended in distilled water and stored in refrigerator at 40°C.

Determination of the concentration of polyhedral suspension

A haemocytometer with improved double Newbauer rulings was used and the counting was done as described by Lewis (1960) under a binocular microscope with 45 x objective. The number of polyhedra per mm^3 was estimated.

Selection of test larvae

In all studies on the nuclear polyhedrosis of P. ricini, except for symptomatology, incubation periods and haemocyte count third instar larvae within 6 to 8 hours after their second ecdysis were used. The symptomatology and incubation period were studied in all instars

except the first. For the haemocyte counts fourth instar larvae were inoculated. Normal larvae had six instars and the larval period lasted for 21 to 24 days.

Care was taken to select larvae of approximately the same age and size for each treatment. This was facilitated by keeping correct records of the dates of hatching of each lot.

Inoculation of caterpillars with the virus

The spot feeding technique deviced by Jacob (1972) was adopted for all the inoculations. The lamina of a middle aged castor leaf was fixed on a thick cardboard with pins with the underside of the leaf facing up. Pieces of paper gum tape, one inch square with circular holes of 6 mm diameter punched in the middle, were pasted over the exposed surface along the periphery of the leaf. Five microlitres of polyhedral suspension containing 0.1 per cent teapol as wetting agent was put into each of the circular exposed leaf disc with a micropipette and the suspension was allowed to dry at room temperature. One larva was confined to each inoculated spot by inverting a penicillin vial over the larva. The petiole of the leaf was kept dipped in

water in a specimen tube. All the instars of larvae completed feeding of the treated area in about 4 hours. Only those larvae which had consumed the treated leaf area completely in 4 hours were taken for the tests and others were discarded. Control larvae were fed similarly except that 5 microlitres of 0.1 per cent teepol only was used instead of the virus inoculum. This method was used in all tests except in studies on the incubation period of the sixth instar larvae. As these larvae were too large to be accomodated in the penicillin vials the following method was used. Leaf discs of 6 mm diameter were cut out and each bit was placed over a wet filter paper kept at the bottom of a plastic jar. The leaf discs were then inoculated with the virus suspension and one larvae each was released over it.

The larvae which had completely ingested the inoculum were transferred to individual plastic containers and supplied with virus free foliage every day.

Diagnosis of dead larvae

The dead larvae were diagnosed by microscopic examination of haemolymph or squashed preparations of tissues for the presence of polyhedra.

Electron micrography

The electron micrography of the virus was done by Dr. Jean R. Adams, Insect Pathology Laboratory, Bettsville, U.S.A.

Susceptibility of different instars of *P. ricini*.

The susceptibility of second, third, fourth, fifth and sixth instar larvae were studied. All larvae were within 6 to 8 hours of their moult to the particular instar. Fifty larvae of each instar were inoculated with a virus suspension of 33×10^7 polyhedra/ml as outlined above. Another set of 50 larvae of each instar treated similarly but without virus inoculum served as control.

The experiments were conducted at room temperature which varied between 24.5° and 30.5°C. The relative humidity during the period ranged from 85 to 92 per cent.

Effect of the virus on the moulting of *P. ricini* larvae.

Fifty larvae each of the third and fourth instars of *P. ricini* were fed with a virus suspension of 33×10^7 polyhedra/ml as described earlier. Another set of 50 larvae of each instar fed on untreated leaves served as control. The larvae in the treated and control group were kept

individually in plastic containers and observations were recorded on the moulting and mortality of the larvae.

Total haemocyte count

Fourth instar larvae within 6 to 8 hours of their third moult were used for this purpose. They were inoculated as already described. Haemolymph samples were drawn at 24, 48, 72, 96, 120, 144 and 168 hours after treatment. Haemocyte of 10 larvae were estimated at each interval. The larvae were immersed in hot water at 55°C to 60°C for 2 to 3 minutes (Jones, 1962). Blood was withdrawn by cutting a proleg on the sixth abdominal segment with a fine scissors into a Thoma white cell pipette upto the 0.5 mark and diluted with 2 per cent versene saline.(Patton and Flint, 1959) upto the 11 mark. This gave a 20 fold dilution of the original volume. The pipette was shaken for several minutes and the first three drops were discarded. A haemocytometer (improved double Neubauer ruling) chamber was filled and the haemocytes were counted at a magnification of 450 x as outlined by Jones (1962). The number of haemocytes per cubic millimeter was calculated with the formula.

$$\frac{\text{Haemocytes in five, 1 mm square x dilution x depth factor}}{\text{Number of squares counted}}$$

The statistical 't' analysis was used for comparing the difference between means.

Effect of alkalies on the polyhedra

The following alkali solutions were used.

Sodium hydroxide, 0.1 and 0.2 per cent.

Potassium hydroxide, 0.1 and 0.2 per cent.

Sodium carbonate, 5 and 10 per cent.

A drop of fairly pure polyhedral suspension was put on a clean microscopic slide and dried in the air. The slides were then dipped in alkali solutions for varying periods and were then examined under a binocular research microscope at magnification of 450 x for the presence or absence of polyhedra.

Thermal inactivation point of the virus

One ml of a fairly purified inoculum containing 33×10^7 polyhedra/ml was taken in thin walled pyrex glass tubes and heated in a water bath maintained at the particular temperature for 10 minutes. The heating time of 10 minutes was maintained when the suspension reached the desired temperature. The tubes were cooled immediately. The virus

suspension was subjected to heat treatments in the range of 60°C - 100°C.

The heated polyhedral inclusion bodies were fed to third instar larvae as described earlier. Fifty larvae were used in each treatment. Two sets of larvae (50 each) were kept as control; one set fed with untreated leaves and the other fed with untreated virus as a check for viral infectivity. Observations on larval mortality and pupation were recorded. The room temperature ranged from 22.5° to 30°C and the relative humidity from 88 to 93 per cent during this period.

Effect of continual exposure of the virus to field temperature

Six 1 ml aliquots of a virus suspension containing 33×10^7 polyhedra/ml were poured into clean sterilized petridishes and they were air dried under on electric fan. The petridishes containing the polyhedra were subjected to heat treatment in a hot air oven for 12, 24, 48, 72, 96 and 120 hours respectively. The hot air oven was adjusted to maintain a constant temperature of 35°C throughout, which approximates to the highest field temperature attained during summer months. On completion of the heat treatment each sample was suspended in one ml of sterile

distilled water containing 0.1% teepol to get the original concentration.

This virus suspension was fed to third instar larvae as described earlier. Fifty larvae were used in each test. After feeding on the treated leaves the larvae were transferred to sterilized glass trough and supplied with fresh leaves. An equal number of larvae were kept as control and fed with untreatedcastor leaves. A similar set of larvae were fed with untreated virus as a check for viral infectivity. The experiment was conducted at room temperature which varied between 22.9° - 29.9°C and a relative humidity of 89 to 93 per cent. Observations were recorded on larval mortality, pupation and adult emergence.

Effect of sunlight on the virus

Six 1 ml aliquots of virus suspension containing 33×10^7 polyhedra per ml were poured into clean and sterilized petridishes and dried under an electric fan. The polyhedra were then exposed outdoors to direct sunlight in open dishes for 12, 24, 48, 72, 96 and 120 hours during December 1974 at Vellayani. After exposure each sample was suspended in 1 ml of sterile distilled water containing 0.1 per cent teepol to get the original concentration.

The virus suspension was fed to third instar larvae as described earlier. In each test 50 larvae were used. On completion of feeding they were transferred to sterilized glass trough and supplied with fresh castor leaves. An equal number of larvae fed on untreated castor leaves served as control. A similar set of larvae were fed with virus not exposed to sunlight as a check for viral infectivity. The experiments were conducted at room temperature ranging between 22.9°C - 29.9°C and relative humidity 89 to 93 per cent.

Observations on larval mortality, pupation and moth emergence were recorded.

Cross infectivity

Cross infectivity of the nuclear polyhedrosis virus of Pericallia ricini to the following species of Lepidoptera were studied.

1. Achoea janata L. (Noctuidae)
2. Spodoptera litura F.(Noctuidae)
3. Diacrisia obliqua Walker (Arctiidae)
4. Euproetis fraterna Moore (Lymantridae)
5. Glyphodes marginata F.(Pyralidae)

Inoculations were made as described earlier with a virus suspension of 33×10^7 polyhedra/ml.

Larvae of *S. litura* and *A. janata* were obtained from pure laboratory cultures and were fed with contaminated castor leaves. Larvae of *E. fraterna*, *D. obliqua* and *G. marginata* were collected from the field and fed with contaminated leaves of castor, cowpea and crape jasmine (*Tabernae montana*) respectively. After feeding on the contaminated leaves the larvae were fed on fresh leaves.

RESULTS

RESULTS

Symptomatology

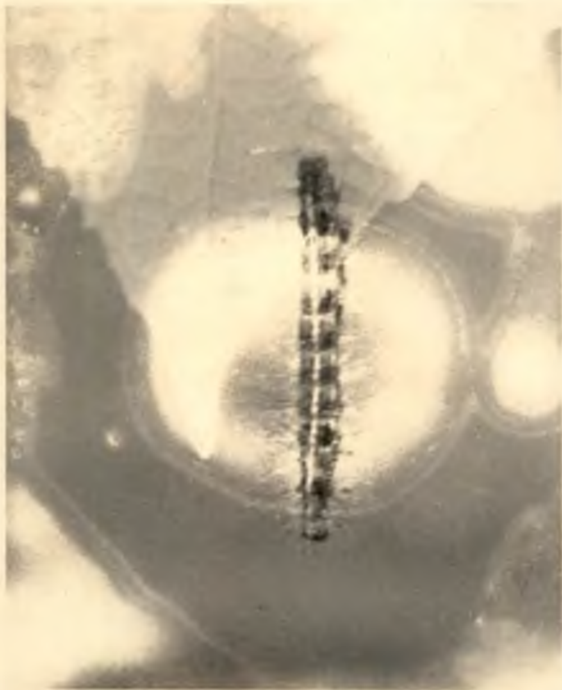
The symptoms of nuclear polyhedrosis infection in the larvae of Pericallia ricini became evident 3 to 4 days after ingestion of the virus. The infected second and third instar larvae assumed a paler colouration than the healthy larvae. The later instars did not exhibit any colour changes in the early stages of viral infection.

The infected larvae became lethargic and showed loss of appetite after 3 to 4 days of ingestion of the virus. They were less responsive to tactile stimuli. The diseased larvae sometimes discharged a dark brown fluid through the mouth. They stopped feeding 2 or 3 days before death. In the advanced stages of infection the cuticle was very fragile which ruptured on the slightest pressure liberating the liquefied body contents containing the polyhedra.

The dead or dying late instar larvae exhibited the typical symptom of hanging head downwards from the top and sides of the container. But most of the second and third instar larvae were found lying dead on the leaf surface or on the bottom of the container. The cadaver darkened very soon and dried upto a dark scale within 24 to 48 hours. No

Fig. 1. Larva of *Pericallia ricini* died of nuclear polyhedrosis showing the rupturing of cuticle.

Fig.2. Larva of *Pericallia ricini* died of nuclear polyhedrosis showing the characteristic hanging position.



pupal mortality was observed.

The body fluid of the infected larvae appeared clear in the early stages but it turned turbid later. The fat body appeared opaque white in colour. The incubation period of the disease was found to vary from 4 to 10 days.

Susceptibility of different instars of larvae

The incubation period and per cent mortality of different instars of larvae are presented in Table 1. It will be seen from the table that the resistance of the larvae to nuclear polyhedrosis infection increased with the age of the larvae. The incubation period was prolonged from 5.24 days in the second instar larvae to 8.2 days in the fifth instar larvae. The second, third and fourth instar larvae were highly susceptible to the virus recording mortalities of 100, 92, and 92 per cent respectively. The fifth instar larvae were less susceptible to virus infection recording a mortality of 72 per cent. The sixth instar larvae showed remarkable resistance to virus infection, the mortality observed being only 8 per cent.

Effect of nuclear polyhedrosis on the larval moulting of *P. ricini*.

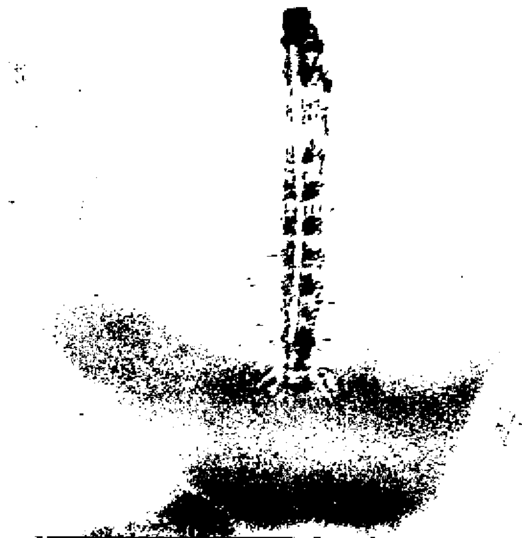
Tables 2A and 2B summarise the observations on the effect of nuclear polyhedrosis on the larval moulting of

Table 1

The incubation period and per cent mortality of *P. ricini* when infected by NPV at different instars.

Stage of larvae treated	Time taken for death (days)		No. of larvae inoculated.	No. of larvae dead		% mortality due to NPV	Mortality in control	
	Range	Mean		Due to NPV	Due to other causes		Due to NPV	Due to other causes
Second instar	4-7	5.24	50	50	..	100	Nil	Nil
Third instar	4-8	5.91	50	46	4	92	"	"
Fourth instar	4-10	7.04	50	46	4	92	"	"
Fifth instar	6-9	8.20	50	36	6	72	"	"
Sixth instar	8	8.00	50	4	..	8	"	"

Fig.3. Per cent mortality of *Pericallia ricini* larvae when infected by NPV at different instars.



AVERAGE NUMBER OF CIRCULATING HAEMOCYTES IN HEALTHY AND NPV INFECTED LARVAE OF *P. ricini* AT DIFFERENT INTERVALS AFTER INOCULATION

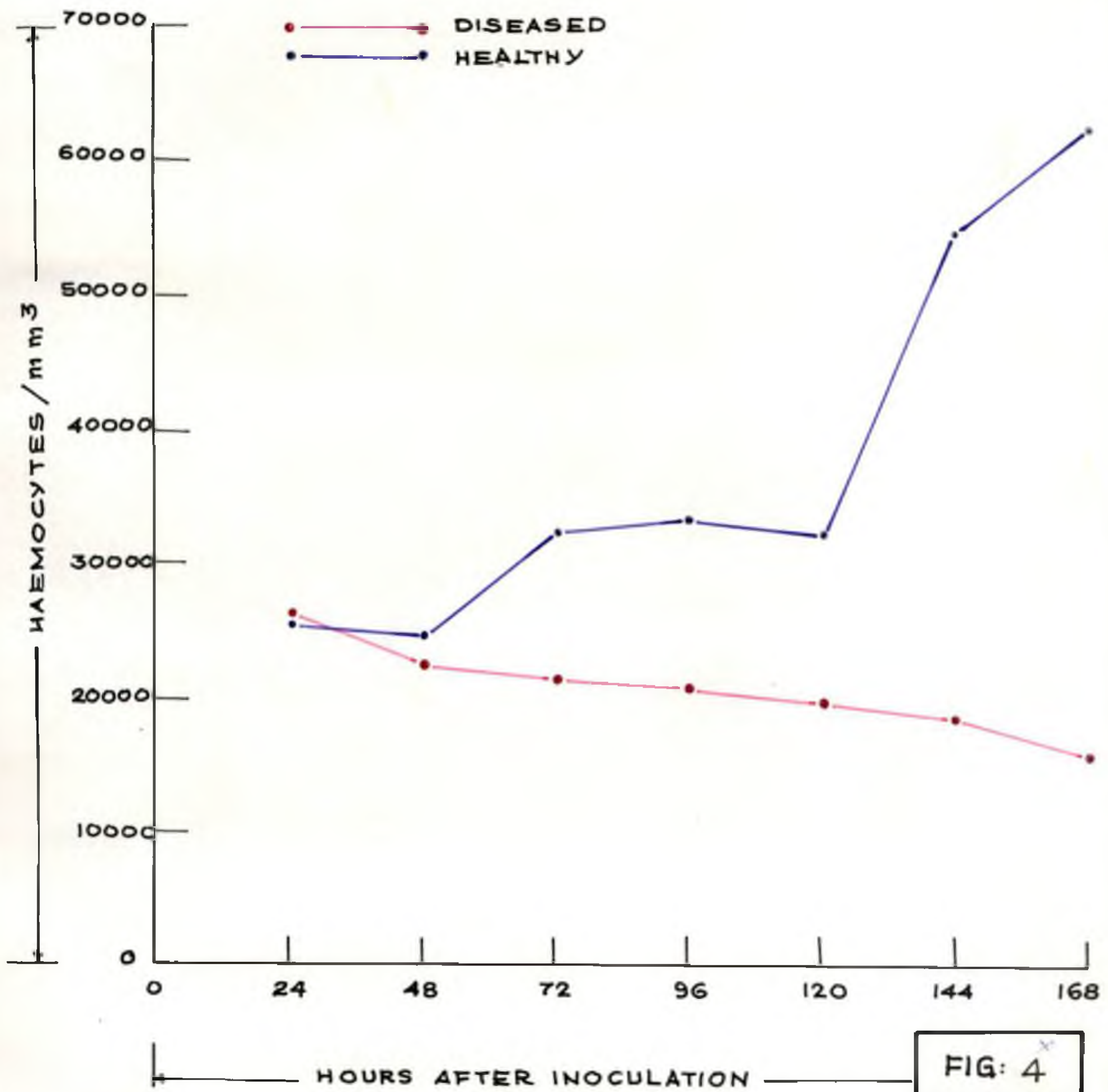


FIG: 4

P. ricini when infected in the third and fourth instar respectively.

Table 2A

Effect of NPV infection on the moulting of *P. ricini* when infected in the third instar.

Treatment	No. of test larvae	Incubation period	No. of larvae moulted to		No. of larvae dead		No. of larvae pupated
			4th instar	5th instar	Due to NPV	Due to other causes	
Inoculated	50	4-9	44	Nil	48*	2	Nil
Control	50	..	50	50	Nil	1	49

* 4 larvae died due to NPV before the first moult.

Table 2B

Effect of NPV on the moulting of P.ricini when infected in the fourth instar.

Treatment	No.of test larvae	Incub-ation period	No.of larvae moulted to		No.of larvae dead		No.of larvae pupa-ted
			5th ins-tar	6th ins-tar	Due to NPV	Due to other causes	
Inoculated	50	5-10	50	6	42	2	6
Control	50	..	50	50	Nil	Nil	50

The results presented show that out of 50 third instar larvae inoculated with the virus 44 larvae underwent the first moult (3rd) on the fourth day and entered the fourth instar. But none of them had undergone the subsequent moulting. In the case of 50 fourth instar larvae inoculated with the virus (Table 2B) all had undergone the first moult (4th) on the fourth day and reached the fifth instar. But only 6 larvae had the second moult (5th) and entered the sixth instar. The third and fourth instar larvae kept as control underwent two moultings during the experimental period.

Total haemocyte count

The average number of circulating haemocytes (THC) in healthy and virus infected larvae of P.ricini is presented in Table 3 and illustrated in Fig. 4

Table 3

Mean number of circulating haemocytes in healthy and NPV infected larvae of *P.ricini*

Post inoculation period in hours	Average number of circulating haemocytes/ mm^3 ± SE		% increase (+) or decrease (-) over healthy
	Healthy larvae	Infected larvae	
24	25500 ± 607.56	26200 ± 643.33	+ 2.64
48	24330 ± 507.73	22300 ± 482.93	- 8.34*
72	32200 ± 568.03	21200 ± 473.76	- 31.05*
96	32750 ± 624.90	20500 ± 657.43	- 37.41*
120	32050 ± 1187.60	19650 ± 465.33	- 41.81*
144	54500 ± 622.70	18500 ± 390.83	- 66.05*
168	62000 ± 863.96	15750 ± 335.40	- 74.59*

* Significant at 5 per cent level.

Fig.4. Average number of circulating haemocytes in healthy and NPV infected larvae of *Pericallia ricini* at different intervals after inoculation.

SUSCEPTIBILITY OF DIFFERENT INSTARS OF *P. ricini* TO NPV

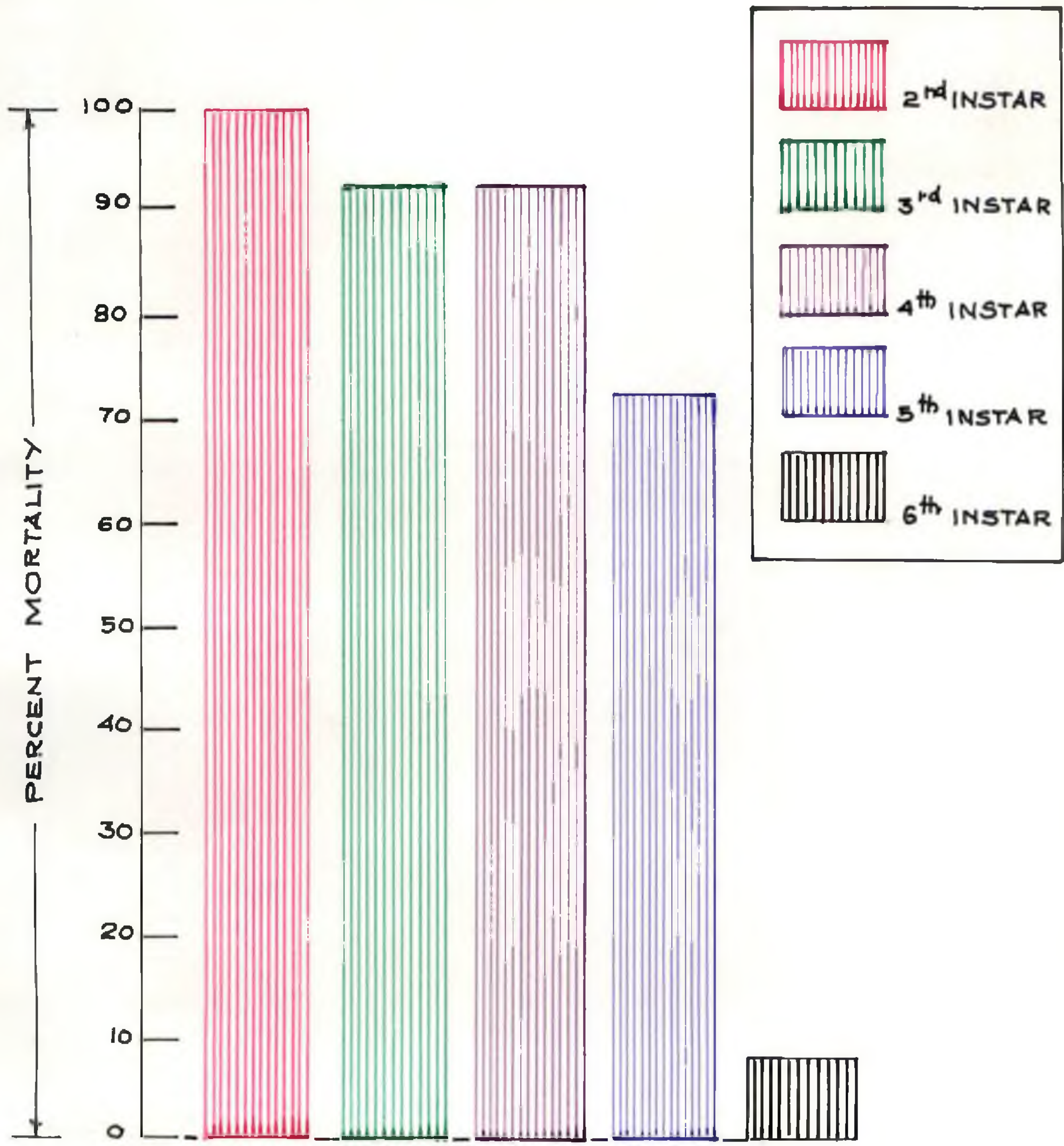
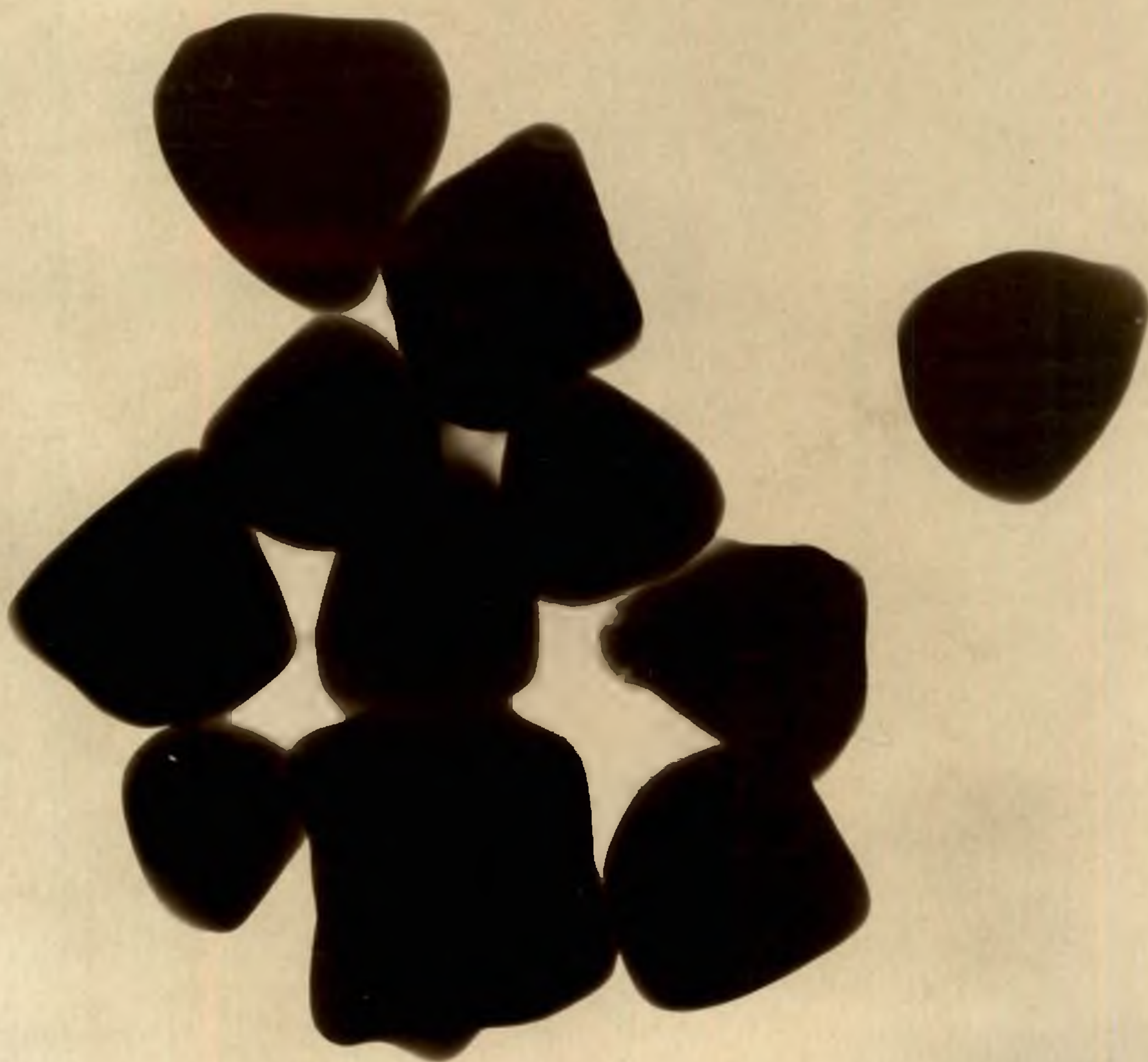


FIG: 3

Fig. 5. Electron micrograph of polyhedra isolated from diseased larvae of *Pericallia ricini* 29675 X.



It is seen that the average number of circulating haemocytes in healthy larvae increased with age except for the slight declines recorded at the premoulting stages at 48 hours and 120 hours after the start of the experiment. In healthy larvae the average number of haemocytes increased from 25500 ± 607.56/mm^3 at 24 hours to 62000 ± 863.96/mm^3 at 168 hours. In the diseased larvae the THC decreased from 26200 ± 643.33/mm^3 at 24 hours reaching the lowest number of 15750 ± 335.40/mm^3 at 168 hours after inoculation.

A comparison of the THC of healthy and diseased larvae at different intervals using 't' test showed that the diseased larvae had significantly (P=0.05) lower number of haemocytes at all intervals except at 24 hours after treatment.

Size and shape of polyhedra

The polyhedra were irregular in shape and many sided with blunt angular corners (Fig. 5).

Table 4
Frequency distribution of diameters of polyhedra of *P. ricini*

Diameters (m/u)	Frequency
936-1056	6
1056-1176	8
1076-1296	19
1296-1416	8
1416-1536	2
1536-1656	2
1656-1776	3
1776-1896	2

The diameter of 50 polyhedra were measured (Table 4) and it ranged from 943.6 m μ to 1829 m μ and averaged 1284.0 $\pm$ 12.48 m μ. Seventy per cent of the polyhedra ranged between 1056 m μ and 1416 m μ in diameter.

Effect of alkali on polyhedra

Table 5 shows the results of treatment of the polyhedra with different alkalies.

Table 5

Dissolution of polyhedra of *P.ricini* in different concentrations of alkali

Time in minutes given for dissolution.	Potassium hydroxide		Sodium hydroxide		Sodium carbonate	
	0.1%	0.2%	0.1%	0.2%	5%	10%
1	+	-	+	-	+	+
2	+	-	+	-	+	-
3	+	-	+	-	+	-
4	+	-	+	-	+	-
5	+	-	+	-	-	-
10	+	-	+	-	-	-
15	-	-	-	-	-	-

(+) Polyhedra present (-) Polyhedra dissolved

It may be seen that 0.2 per cent KOH or NaOH dissolved the polyhedra within one minute while 0.1 per cent solution of either alkali required more than 10 minutes to produce the same effect. With 5 per cent or 10 per cent Na_2Co_3 the dissolution of polyhedra was achieved in 5 minutes and 2 minutes respectively.

Thermal inactivation point

The results of the bioassay of the heated virus against the third instar larvae of P. ricini are furnished in Table 6 and illustrated in Fig. 6

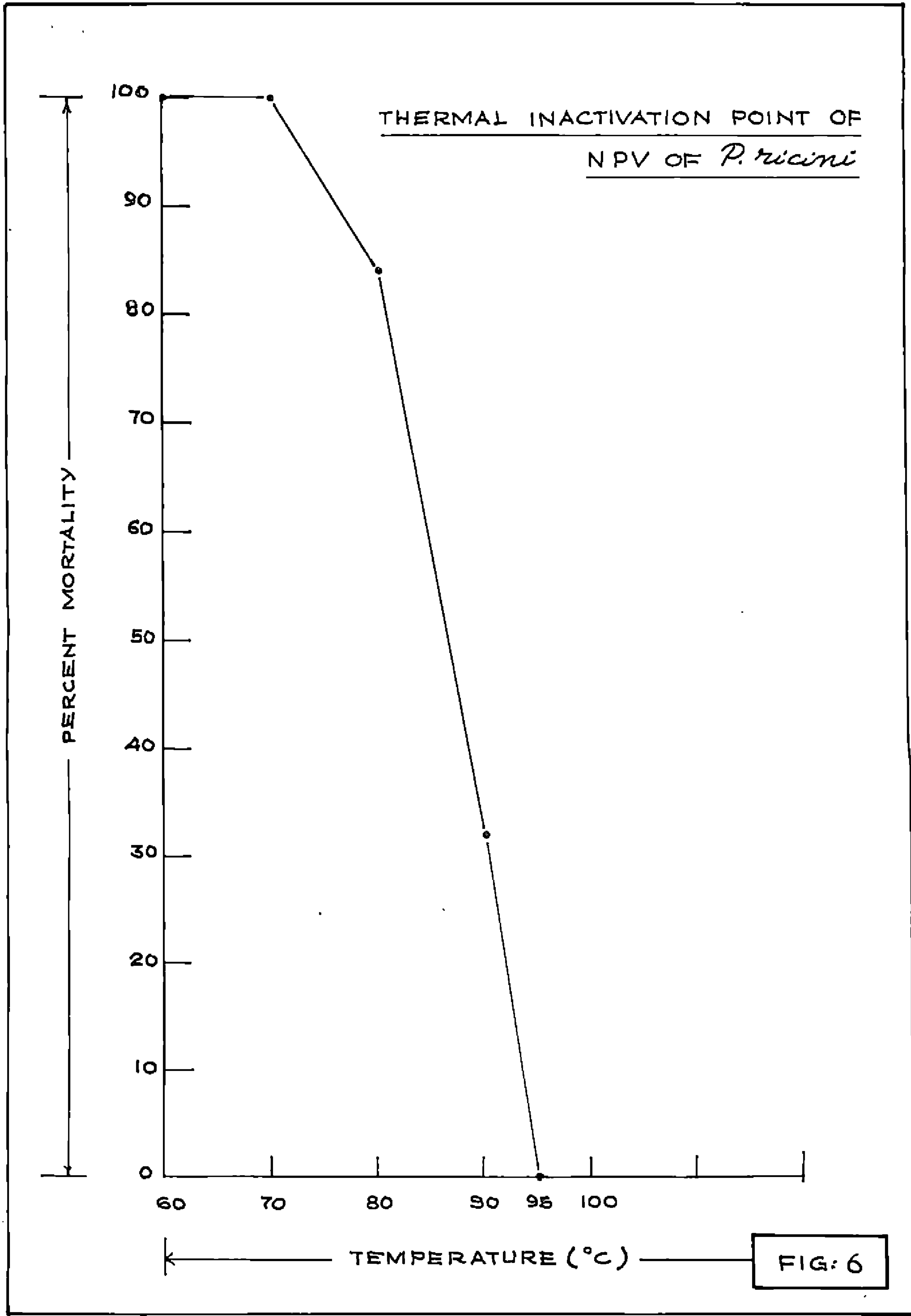
Table 6

Thermal inactivation point of NPV of P.ricini

Temperature. °C	Time taken for death (days)		% mortality due to NPV	% mortality due to other causes	% pupation
	Range	Mean			
60	5-9	6.92	100	Nil	Nil
70	5-10	7.28	100	"	"
80	5-10	7.43	84	"	16
90	7-11	9.62	32	4	64
95	..	..	Nil	4	96
100	..	..	Nil	Nil	100
Control	..	..	Nil	Nil	100
Control (treated)	4-9	6.0	100	Nil	Nil

The data presented show that there was 100 per cent mortality among the larvae when fed with polyhedra

Fig.6. Thermal inactivation point of the NPV of *Pericallia ricini*.


THERMAL INACTIVATION POINT OF
NPV OF P. ricini
PERCENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
60
70
80
90
95
100
TEMPERATURE (°C)
FIG: 6

subjected to heat treatment at 60°C or 70°C for 10 minutes. The larval mortality was reduced progressively as the heat treatment was increased from 80 to 90°C. There was no mortality due to nuclear polyhedrosis in the larvae treated with virus heated to 95°C and 100°C and all the larvae except those dead due to other causes pupated normally.

Effect of continual exposure of the virus to field temperature

Observations on the incubation period, larval mortality and pupation of the third instar larvae fed with heat treated polyhedra are furnished in Table 7.

The results show that heat treatment at 35°C upto 96 hours did not significantly affect the infectivity of the virus. But exposure for 120 hours resulted in substantial lose of infectivity. The larval mortality was reduced from 100 per cent with polyhedra after 12 hours of heat treatment to 8 per cent with those after 120 hours heat treatment. 84 per cent of the larvae fed with 120 hours heat treated polyhedra pupated normally. The incubation period of the disease also showed an increase with the period of heat treatment of the virus. Thus it increased from 6.1 days for the 12 hours treatment to 10.3 days for the 120 hours treatment.

Table 7

Effect of continued exposure to field temperature on the infectivity of NPV of *P. ricini*.

Duration of exposure to field temperature (hours)	Time taken for death (days		No.of larvae inoculated.	No. of larvae dead		% mortality due to NPV	% pupation
	Range	Mean		Due to NPV	Due to other causes.		
12	3-9	6.1	50	50	Nil	100	Nil
24	5-9	6.2	50	48	2	96	"
48	4-10	6.8	50	48	2	96	"
72	5-10	7.8	50	46	4	92	"
96	5-12	9.2	50	44	6	88	"
120	10-11	10.3	50	6	2	12	84
Control	..	..	50	..	1	Nil	98
Control (treated)	4-9	6.1	50	50	Nil	100	Nil

Effect of sunlight on the infectivity of the virus

The data on the incubation period, larval mortality and pupation of the third instar larvae inoculated with polyhedra exposed to sunlight for different periods are recorded in Table 8.

Table 8

Effect of sunlight on the infectivity of the NPV of P. ricini.

Duration of exposure to sunlight (hours)	Time taken for death (days)		No. of larvae inoculated.	No. of larvae dead		% mortality due NPV	% Pupation
	Range	Mean		Due to NPV	Due to other causes		
12	4-9	6.5	50	50	..	100	Nil
24	4-10	7.0	50	48	2	96	"
48	5-10	7.5	50	48	2	96	"
72	6-12	9.9	50	46	4	92	"
96	7-12	10.3	50	18	8	36	48
120	9-11	10.5	50	4	4	8	84
Control	..	..	50	Nil	Nil	Nil	100
Control (treated)	4-9	6.2	50	49	1	98	Nil

It is evident that the infectivity of the virus was unaffected by exposure to sunlight upto a period of 72 hours but further exposure to sunlight reduced its infectivity. The mortality of the larvae inoculated with the virus subjected to 96 hours and 120 hours exposure was 36 and 8 per cent respectively. The incubation period of the disease was prolonged from 6.5 days in the treatment with polyhedra after 12 hours exposure to 10.5 days with polyhedra subjected to 120 hours exposure to sunlight.

Cross infectivity

Table 9

Cross infectivity of NPV of P.ricini to other species of Lepidoptera.

Test insect	Stage of larvae at inoculation.	No.of larvae inoculated.	Mortality		Infectivity
			Due to NPV	Due to other causes	
Achoea janata	Third	30	Nil	Nil	- ve
Spodoptera litura	Fourth	30	"	"	- ve
Euproctis fraterna	Third	50	"	5	- ve
Glyphodes marginata	Third	20	"	Nil	- ve
Diacrisia obliqua	Third	50	44	6	+ ve

It will be seen from the results presented in Table 9 that the NPV of P.ricini was cross transmissible to the larvae of Diacrisia obliqua (Arctiidae) recording a mortality of 88 per cent. But the virus was not infective to the other larvae tested viz. A. janata, S. litura, E. fraterna and G. marginata and they pupated normally and adults emerged except for those died due to other causes.

DISCUSSION

DISCUSSION

The objectives of the present investigation have been to collect basic information on the host-pathogen relationships between the larvae of *Pericallia ricini* and its nuclear polyhedrosis virus to assess the suitability of this pathogen for field application. Studies were conducted on the symptomatology, larval susceptibility, effect of virus on moulting of larvae, changes in the number of circulating haemocytes in the virus infected larvae, nature of causative agent, effect of temperature and sunlight on the infectivity of the virus, and cross transmissibility of the virus to other species of Lepidoptera.

The virus infected *P. ricini* larvae were found to exhibit all the characteristic symptoms of nuclear polyhedrosis infection as reviewed by Aizawa (1963) and Smith (1967). Some of the early instar larvae were found to die without showing any characteristic symptom of virus infection. Similar observations have been made by Harpaz and Zlotskin (1965) in *Heliothis peltigera* and Jacob (1972) in *Heliothis armigera*.

Observations made on the susceptibility of different larval instars of *P. ricini* to nuclear polyhedrosis infection

revealed that the susceptibility of the larvae decreased as they advanced in age. Decrease in susceptibility of older larvae to nuclear polyhedrosis virus has been reported by Tanada (1956) in Pseudaletia unipuncta, Morris (1962 a) in Lambdina fiscellaria somniaria, Stairs (1965 a) in Malacosoma disstria, Doane (1967) in Porthetria dispar, Jacob and Subramaniam (1972 b) in the larvae of Spodoptera litura and Lathika and Jacob (1974 b) in Spodoptera mauritia. This increase in resistance associated with the growth of the larvae was regarded by some authors as 'maturation immunity'. Ignoffo (1966 a) attributed this partly to the normal increase in body weight which might serve to dilute a constant virus dose.

The results of experiments to study the effect of virus infection on moulting of third and fourth instar larvae of P. ricini showed that the virus infection did not influence the first moulting which occured within four days of inoculation in the third and fourth instar larvae, but it inhibited the subsequent moulting which should occur on the 7th or 8th day after inoculation. Vago (1950, 1956) observed that nuclear polyhedrosis infection at the

incipient stages of pathogenesis caused moulting disturbances. Jacob and Subramaniam (1974) observed that nuclear polyhedrosis infection inhibited moulting in Spodoptera litura larvae in the later stages of the disease. Rabindra and Subramaniam (1974) also made a similar observation in the NPV infected larvae of Heliothis armigera.

The hormonal control of moulting and metamorphosis is now well established. Morris (1970) reported that there was an apparent reduction in the amount of neuro-secretion in the brain after NPV infection in Lambdina fiscellaria somniaria and Orgyia pseudatsugata. Thus it is possible that in the present instance also, the infection of the virus interfered with the neurosecretory activity leading to consequent moulting disturbances. Moreover, the virus also causes inactivation of the hypodermis making it non-responsive to activation by prothoracic gland for the deposition of a new cuticle which initiates the moulting process as reported by Jacob (1972).

Observations recorded on the variations in the number of circulating haemocytes showed that in the healthy larvae the THC increased with age interrupted by slight

declines at premoulting stages. In the present studies the healthy larvae were in the premoulting stages at 48 hours and 120 hours of the start of the experiment. Similar observations have been made by Patton and Flint (1959) in the nymphs of American cockroach, Periplaneta americana L., and Nittono (1960) in the silk worm Bonbyx mori. Jacob (1972) also observed a sharp decline in THC at the premoulting stages and an increase thereafter in the healthy larvae of S. litura.

The total haemocyte count showed no significant difference between the healthy and infected larvae at 24 hours after inoculation. Similar observations have been reported in wax moth, Galleria mellonella harvae (Shapiro, 1967) and in the larvae of S. mauritia (Lathika and Jacob, 1974 c). However Jacob (1972) observed a significant increase in the number of haemocytes in the diseased larvae of S. litura 24 hours after inoculation compared to healthy larvae of the same age. In the present studies, it was also observed that the THC steadily decreased from 48 hours onwards in the infected larvae. Shapiro et al. (1969) also noted a drastic reduction of haemocytes in Heliothis zea larvae after exposure for three days to a high dose of nuclear polyhedrosis virus but not

in healthy larvae or in larvae exposed to a low dose of the virus. Rabindra and Subramaniam (1974) reported a significant reduction of haemocytes in the NPV infected larvae of H. armigera from the third day of infection. The destruction of haemocytes and interference in the mitotic division of the cells by the virus infection may account for the decrease in total haemocyte count of the diseased larvae.

The diameter of polyhedra of P. ricini was found to vary from 943.6 m/u to 1829.0 m/u, the average being 1284.0 ± 12.48 m/u. According to Smith (1967) the diameter of polyhedra varied generally from 0.5 to 15/u depending on the host species. Jacob and Subramaniam (1972 a) reported that the average diameter of polyhedra of Amsacta albistriga was 1.17/u . Lathika and Jacob (1974 b) reported that the polyhedra from Spodoptera mauritia measured on an average 1.13 /u in diameter. Jacob et al.(1972) reported that the virus particles in the polyhedra of P. ricini was rod shaped and were arranged singly or in groups. These characteristics indicate that the pathogen belongs to the group Borrelinavirus causing nuclear polyhedroses in insects.

The dissolution of polyhedra in alkaline solutions is well established and the degree of resistance to alkaline treatment vary with the polyhedra from different polyhedroses. In the present experiment it was found that 0.2 per cent KOH or NaOH dissolved the polyhedra in one minute while 0.1 per cent solution of either alkali required more than 10 minutes to produce the same effect. Dissolution of polyhedra in 5 per cent and 10 per cent Na_2Co_3 was achieved in 5 minutes and 2 minutes respectively. Day _et_ _al_.(1963) reported that the polyhedra from Australian pasture caterpillar, _Pterolocera_ _amplicornis_ required 60 minutes to dissolve in 4 per cent Na_2Co_3 at 56°C. Brown and Swaine (1965) reported that the polyhedra of _Spodoptera_ _exempta_ dissolved quickly in 0.2 per cent NaOH while 2 per cent Na_2Co_3 was ineffective in dissolving the polyhedra even after 30 minutes. Lathika and Jacob (1974 b) reported that 0.2 per cent KOH or NaOH and 10 per cent Na_2Co_3 dissolved the polyhedra of _Spodoptera_ _mauritia_ in less than 2 minutes while 0.1 per cent KOH or NaOH and 5 per cent $Na_2 Co_3$ produced such an effect in 3 to 10 minutes. Jacob and Thomas (1974) observed that the polyhedra of _Diacrisia_ _obliqua_ dissolved in 0.1 per cent NaOH or KOH in less than 2 minutes while 0.2 per cent NaOH or KOH produced the same effect in one

minute. But such dissolution of polyhedra with 5 and 10 per cent $Na_2 Co_3$ was achieved only after 30 and 35 minutes respectively. The results of the present experiment suggest that the polyhedra of P. ricini resembled closely those from other lepidopterous larvae in their reaction to weak solutions of KOH or Na OH but were less resistant to 5 per cent and 10 per cent $Na_2 Co_3$. These results also point out that quick and effective sterilization of glass wares and other equipments could be achieved by treatment with 0.2 per cent KOH or NaOH or 10 per cent Na_2Co_3 for few minutes.

It is evident from the results obtained that TIP of the nuclear polyhedrosis virus of P. ricini when heated for 10 minutes lie between 90° and 95°C. This exceeds the general limit of 80°C reported for other inclusion viruses (Bergold, 1958; Aizawa, 1963; Huger, 1963). The comparatively high TIP between 90° and 95°C has also been reported for nuclear polyhedrosis viruses of Prodenia litura (Pawar and Ramakrishnan, 1971 b), and Spodoptera mauritia (Lathika and Jacob, 1974 a). Stuermer and Bullock (1968) reported that the NPV of Heliothis withstood exposure to 60°C for 2 hours and was completely inactivated

at 93.3°C in one hour. The present findings indicate that the NPV of P. ricini is relatively thermostable than most other inclusion viruses studied but is less heat tolerant than Heliothis virus.

Observations made on the effect of continual exposure of the polyhedra to a temperature of 35°C showed that the virus remained highly infective upto 96 hours. It has been reported that continual exposure to higher field temperatures (35°-45°C) might affect viral stability and virual multiplication. (Bird, 1955; Thompson, 1959; Ignoffo, 1966 b). However, Morris (1971) found that heating the nuclear polyhedrosis virus of Lambdina fiscellaria somniaria at 45°C for 200 hours did not affect its infectivity. The present observations indicate that the nuclear polyhedrosis virus of P. ricini was less heat tolerant than the NPV of Lambdina fiscellaria somniaria.

The experiment to find out the effect of sunlight on the infectivity of the virus showed that it could withstand exposure to sunlight for 72 hours without much loss of infectivity. The infectivity of the virus was considerably reduced after 96 hours exposure recording a larval mortality of only 36 per cent. The virus was

almost non-infective after 120 hours exposure to direct sunlight, the mortality recorded being only 8 per cent. Lathika and Jacob (1974 a) observed that the NPV of Spodoptera mauritia retained its infectivity upto 72 hours of exposure to sunlight but was almost non-infective after 96 hours exposure to sunlight.

UV radiation in sunlight has been suggested as one of the factors responsible for inactivation of field applied viruses. Hirt et al. (1960) and Turner and Kaplan (1965) reported that sunlight-UV although not directly responsible might indirectly inactivate or catalyse reactions which resulted in viral instability and loss of activity. Bullock (1967) found that Heliothis virus applied to cotton foliage lost most of its infectivity after one day and this was attributed partly to ultraviolet rays. Cantwell (1967) observed that the NPV of Trichoplusia ni was inactivated by exposure on multipore-filters to direct sunlight for 3 hours. Morris (1971) reported that the virus of Lambdina fiscellaria somniaria retained only 11 per cent infectivity after 35 hours of exposure to sunlight. He also found that UV radiation of predominantly 3600°A was only slightly viricidal implying that UV radiation alone was not sufficient to inactivate the virus. The present observations

on the NPV of P. ricini and that by Lathika and Jacob (1974 a) on the NPV of S. mauritia indicate that under tropical conditions viruses may withstand exposure to sunlight for comparatively longer periods.

A comparison of the data on the effect of sunlight and that of continual exposure to normal field temperature show that after exposure for 96 hours of heat treatment the virus was still highly infective while it retained only some infectivity after exposure to sunlight for the same period. This indicate that under field conditions temperature alone may not be the factor responsible for inactivation of the virus but temperature along with other factors like UV radiation present in the sunlight may be causing the deactivation.

The cross transmission studies proved that the nuclear polyhedrosis of P. ricini was transmissible to Diacrisia obliqua which belongs to the same family Arctiidae. Tanada (1954) also obtained successful cross transmission of the NPV of Colias philodice eurytheme to Pieris rapae and vice versa, both hosts belonging to the same family. Morris (1964) observed that the virus from Caripeta devastata (Geometridae) could be cross transmitted

to the larvae of Lambdina fiscellaria somniaria and Lambdina fiscellaria lugubrosa (Geometridae). In the present experiments attempts of cross transmission of the virus to other species of Lepidoptera viz. Spodoptera litura, Achoea janata (Noctuidae), Euproctis fraterna (Lymantridae) and Glyphodes marginata (Pyralidae) gave negative results. This may be due to the high degree of host specificity which is a characteristic of most insect viruses.

SUMMARY

Detailed investigations were carried out on the nuclear polyhedrosis of the black hairy caterpillar, Pericallia ricini (Arctiidae) covering symptomatology, larval susceptibility, effect of the virus on the larval moulting, changes in the number of circulating haemocytes in the infected larvae, nature of causative agent, effect of temperature and sunlight on the infectivity of the virus and the cross infectivity of the virus to other species of Lepidoptera.

The virus infected larvae exhibited all the typical symptoms of nuclear polyhedrosis infection as reported from other lepidopterous larvae. The larvae infected in the early instars appeared paler and thinner. The infected larvae showed loss of appetite and became sluggish. The dead or dying larvae showed the characteristic symptom of hanging head downwards from the top of the containers with the aid of prolegs. In the advanced stages of infection the cuticle became fragile and it ruptured liberating the liquefied body contents.

The susceptibility of the larvae to virus infection decreased with increase in the age of the larvae. The average

incubation period varied from 5.24 days for the second instar to 8 days for the sixth instar larvae.

The virus infection inhibited moulting in the later stages of the disease.

No significant difference was observed in the THC of healthy larvae and virus infected larvae upto 24 hours after inoculation; there was significant reduction in the THC of diseased larvae after 24 hours of inoculation.

The polyhedra varied in size and shape and measured on an average 1284.0 ± 12.48 mμ in diameter. The virus particles were rod shaped and occurred singly and in bundles.

The polyhedra dissolved completely in 0.2 per cent NaOH or KOH in one minute while 0.1 per cent solution of NaOH or KOH required more than 10 minutes to produce such an effect. Five per cent and 10 per cent solutions of Na_2CO_3 required 5 minutes and 2 minutes respectively to bring about complete dissolution of the polyhedra.

A comparatively high TIP between 90° and 95°C was observed for the virus when heated for 10 minutes.

The virus retained its infectivity upto 96 hours of exposure to 35°C but lost most of its infectivity after exposure for 120 hours.

The virus could withstand exposure to direct sunlight for 72 hours without loss of infectivity but the infectivity was reduced substantially after 96 hours of exposure. It was almost non-infective after 120 hours of exposure.

This virus was found to be cross transmissible to *Diacrisia obliqua* (Arctiidae) but not to *Achoea janata*, *Spodoptera litura* (Noctuidae), *Glyphodes marginata* (Pyralidae) and *Euproctis fraterna* (Lymantridae).

SUMMARY

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