NUCLEAR POLYHEDROSIS OF *Opisina arenosella* WLK. (= *Nephantis serinopa* MEYR:) (CRYPTOPHASIDAE: LEPIDOPTERA) AND ITS UTILITY FOR THE CONTROL OF THE PEST

BY
BABU M. PHILIP

THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE
DOCTOR OF PHILOSOPHY
KERALA AGRICULTURAL UNIVERSITY
FACULTY OF AGRICULTURE

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, TRIVANDRUM

1985

Dedicated to

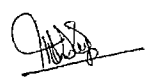
my father Late Sri. [illegible] Philip

and

my mother Smt. Saramma Philip

DECLARATION

I hereby declare that this thesis entitled "Nuclear polyhedrosis of Opisina arenosella Wlk. (= Nephantis serinopa Meyr:) (Cryptophasidae: Lepidoptera) and its utility for the control of the pest" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar titles of any other University or Society.



BABU M. PHILIP

Vellayani,
15th June 1985

C E R T I F I C A T E

Certified that this thesis entitled "Nuclear polyhedrosis of Opisina arenosella Wlk. (= Neohantis serinopa Meyr:) (Cryptophasidae: Lepidoptera) and its utility for the control of the pest" is a record of research work done independently by Sri.BABU M. PHILIP 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.

Dr.N. MUHANDAS
Chairman
Advisory Committee
Professor and Head of Department
of Entomology.

Vellayani,
15th June 1985.

APPROVED BY

CHAIRMAN

Dr. N. MOHANDAS

MEMBERS

Dr.K.P.VASUDEVAN NAIR

Dr. A.M. ASHIY

Dr. JAMES MATHEW

SRI.K.K.RAVEENDRAN NAIR

ACKNOWLEDGEMENT

The author wishes to place on record his deep sense of gratitude to Dr.N.Mohandas, Professor and Head of the Department of Entomology, College of Agriculture, Vellayani, Trivandrum and Chairman of the Advisory Committee for the valuable guidance throughout the course of this investigation and for the help rendered in the preparation and presentation of this thesis. His thanks are also due to Dr.K.P. Vasudevan Nair, Associate Professor, College of Agriculture, Vellayani, Trivandrum for his valuable guidance and for critically reading the manuscript of this thesis.

He is grateful to Dr.M.M.Koshy, Professor, Research Co-ordination, College of Agriculture, Vellayani, Trivandrum for critically reading the manuscript. His thanks are also due to Dr.James Mathew, Associate Professor(Plant Pathology) Regional Agricultural Research Station, Kumarakom, Kottayam and Sri.K.K.Raveendran Nair, Associate Professor (Nematology), College of Agriculture, Vellayani, Trivandrum for their valuable suggestions.

The guidance rendered by Dr.P.Sreeswathy, Associate Professor of Agricultural Statistics, College of Agriculture, Vellayani, Trivandrum in the statistical analysis of the data is gratefully acknowledged.

The author records his immense gratitude to Sri.Abdul Hameed, Associate Professor, Department of Agricultural Chemistry and Soil Science for his sincere help in photography.

The author expresses his immense gratitude and indebtedness to Dr.(Miss)M.A.Arnold, MIBiol, Institute of Virology, Oxford, England for the electron micrography of the virus.

He also acknowledges the help and co-operation rendered by his colleagues and staff, Department of Entomology, College of Agriculture, Vellayani in the conduct of this investigation and preparation of the thesis.

The financial assistance rendered by the Indian Council of Agricultural Research, New Delhi for carrying out this investigation is duly acknowledged.

He also acknowledges the help and co-operation rendered by his wife Smt.Sheela Paul and his daughter Miss. Mala Mary Philip and his relatives in the conduct of this investigation and preparation of the thesis.

The author expresses his immense gratitude and indebtedness to Dr.S.Sulochana, Professor of Microbiology, College of Veterinary and Animal Science, Mannuthy, Trichur for the safety studies of the virus.

He also acknowledges Dr.N.Sadanandan, Dean, Faculty of Agriculture, Kerala Agricultural University for providing all facilities for this study.

BABU M. PHILIP

C O N T E N T S

---:0:---

LIST OF TABLES

(contd..)

LIST OF TABLES (CONTD.)

LIST OF ILLUSTRATIONS

(contd..)

LIST OF ILLUSTRATIONS (CONTD.)

FIGURE NO.

(contd..)

LIST OF ILLUSTRATIONS (CONTD.)

FIGURE NO.

(contd..)

LIST OF ILLUSTRATIONS (CONTD.)

FIGURE NO.

(contd..)

LIST OF ILLUSTRATIONS (CONTD.)

FIGURE NO.

INTRODUCTION

INTRODUCTION

The black headed caterpillar, Opisina arenosella Wlk. (=Nephantis serinopa Meyrick) is a serious pest of coconut (Cocos nucifera L.) in many parts of South India, especially along the coastal tracts. The slender caterpillars having prominent black heads live under the leaves in galleries made of silk and excreted matters and feed on the green tissues. Infested trees are easily recognised by dried up patches in the fronds and gradual withering.

The pest occurs in serious proportions in summer months. During the remaining part of the year the pest population is suppressed by larval parasitoids like Bracon brevicornis, Wesm., Apanteles taragamae Vier., and Perisierola nephantidis Muesebreck. and pupal parasitoids like Trichospilus pupivora Ferr. and Brachymeria spp. Though insecticidal treatment is recommended in heavily infested gardens, the use of toxic chemicals will have adverse effect on the natural enemy complex, and the very operation of insecticidal spraying on the crown is hazardous.

The pest outbreaks occur in summer months and during the period the parasitoid population normally remains at a very low level. Application of insecticides may wipe out the parasitoid population completely, often

causing a resurgence of the pest incidence in due course. Any microbial insecticide which does not kill the parasitoids will preserve the small number of parasitoids available for subsequent build up when favourable conditions return.

Among the different group of microbial insecticides, the viruses have a high potential for the control of pests. The use of such pathogens have gained importance in the development of ecologically oriented crop pest management programmes (Falcon, 1973). Among the different groups of insect viruses the nuclear polyhedrosis virus and granulosis virus (Baculoviridae) offer great potential for the control of arthropods of importance in agriculture and forestry and this view is endorsed by several workers (Summers et al., 1975; Kinsely, 1975). Baculoviruses are considered suitable for this purpose because of their probable efficiency and safety in use, good storage properties and relative ease of production (Kinsely, 1979).

In India the exploitation of the above technology is yet to gain momentum. A potent virus causing heavy mortality in the natural population of O. arenosella was reported by Philip et al., (1982). But the much

needed basic informations for judging the suitability of this pathogen for field control of the pest were lacking. Hence detailed investigations were taken up for studying the following aspects of the pathogen: symptomatology, susceptibility of different larval instars to the virus, morphology of the pathogen, histopathology, mode of transmission to the progeny, cross infectivity to other species of lepidoptera, persistence of the virus on coconut foliage, effect of physical factors on the virulence of the virus, safety of the pathogen to non-target organisms and efficacy of the virus in controlling the pest in field.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A brief review of literature on nuclear polyhedrosis viruses relevent to the aspects covered in the present studies is presented below.

2.1. Symptomatology

The symptoms caused by nuclear polyhedrosis virus infection vary widely in different insect species. General symptoms observed on lepidopterous larvae infected with the pathogen were reviewed by Aizawa (1963) and Smith (1967). The skin of the infected larvae became oily followed by a colour change or development of a mottled appearance. As the disease advanced, the larvae became lethargic and they lost appetite and stopped feeding. The cuticle became increasingly fragile and finally the body wall ruptured liberating the liquefied body content. In the later stages of the disease, the larvae showed a tendency to seek the highest point available and hang head downwards. The incubation period of the disease varied from three to 21 days. Subsequent workers also reported symptoms similar to those described above (Patel et al., 1968; Jacob and Thomas, 1972; Lathika and Jacob, 1974a;

McCollom and Reed, 1974; Pawar and Ramakrishnan, 1974, Rabindra et al., 1975; Nair and Jacob, 1976; Jacquemard and Delattre, 1977; Legacion and Gabriel, 1978).

But the death of the infected larvae occurring without the above typical symptoms was observed in Heliothis peltigera Denis and Schiff. (Harpaz and Zlotskin, 1965); and in H. armigera Hubner (Jacob, 1972). Morris (1970) reported precocious development of antennae, mouth parts and partial fusing of ocelli in the larvae of Orgyia pseudotsugata Mc Dunn. and Lambdina fiscellaria somniaria Hulst when infected with nuclear polyhedrosis in the fourth instar. Jacob et al., (1973) found that the cadaver of Spodoptera mauritia Boisduval turned black in a short time after death. Nuclear polyhedrosis infection inhibited moulting in Spodoptera litura Fabricius (Jacob and Subramaniam, 1974) and in Heliothis armigera (Rabindra and Subramaniam, 1974). Rabindra et al., (1975) conducted detailed studies on polyhedrosis virus infection in the larvae of Chrystodexis chalcites Esp: (Plusia chalcytes) and found that it caused hypertrophy of the nuclei of the midgut epithelial cells followed by rupturing of integument. Nair and Jacob (1976) observed that the nuclear polyhedrosis virus of Pericallia ricini Fab. caused the discharge of dark brown fluid through the mouth of their host. According to Jacquemard and Delattre (1977) the symptoms of virus infection

on Dinaropsis watersi Roth which usually appeared on the theird day after consumption of contaminated food were similar among different instars though the speed of mortality and per cent mortality varied in them. None of the larvae pupated and mortality was usually complete in six days. Legacion and Gabriel (1978) observed that some larvae of Spodoptera litura which survived the infection of nuclear polyhedrosis gave rise to forms intermediate between larvae and pupae as well as adults with malformed wings. Rao and Amonakar (1980) found that the flaccidity normally seen associated with polyhedrosis in Lepidoptera was not seen associated with a nuclear polyhedrosis of Amsacta moorei Moore.,

2.2. Morphology of the polyhedra:

The size and shape of polyhedra very considerably in different insects and also in different tissues of the insect. According to Gerhenson (1960) it was the virus that controlled the shape of the polyhedra rather than the host cell. Tanada (1960) recorded a larger polyhedra in Spodoptera mauritia which varied in diameter from 1.07 to 3.22 /u.

Bergold (1963) pointed out that in silkworm Bombyx mori Linnaeus the prevailing type of polyhedra were dodecahedra, where as those of Lymantria monacha Linnaeus consisted

mostly of tetrahedra. In the case of a nuclear polyhedrosis of Barathra brassicae Linnaeus the diameter varied from 0.8 to 2.7 /u (Ponson and DeJong, 1964).

The diameter of polyhedra was reported to vary generally from 0.5 to 15 /u among different species of insects (Smith, 1967). Hunter and Hall (1968) found that the polyhedra of Spodoptera exigua Hubner varied in diameter between 1 to 6/u with an average of 2.5 /u.

Jacob and Subramaniam (1972a) observed that the diameter of polyhedra from Amsacta albistriga Wlk. ranged from 0.7 to 1.70 /u with an average of 1.17/u and in the case of Heliothis armigera and S. litura the polyhedral bodies were irregular in shape and measured 0.5 to 1.4 /u in diameter with a mean of 0.89 /u and 1.86 /u with a range of 1.2 to 2.8 /u.

Lathika and Jacob (1974b) reported that the polyhedra from S. mauritia had an average diameter of 1.3 /u with a range of 0.44 to 1.76 /u. Electron microscopic studies on polyhedra of Pericallia ricini Feb. by Nair and Jacob (1976) showed that they were irregular in shape and varied considerably in size with a diameter ranging from 943.6 m /u to 1829 m /u with an average of 1284.6 $\pm$ 12.48 m /u.

2.2.1. Morphology of the virus particle

In Tipula paludosa Mg., Smith and Xeros (1954) observed that the virus rods were arranged in lines. The virus rods occurred singly in H. armigera (Bergold and Ripper, 1957). Bergold (1963) recorded upto 18 rods in one bundle in the polyhedra of Lymentria monacha.

In general, the nuclear polyhedrosis virus particles were reported to be rodshaped and were enclosed in an outer developmental membrane and an inner intimate membrane. Though more than one virus particle was present in a developmental membrane, an intimate membrane contained only one viral unit (Smith, 1967). Ten to 100 virions were found embedded singly or in bundles within each polyhedra and each rod averaged 400 x 80 m μ in size (Ignoffo, 1968). Jacob and Subramaniam (1972b) reported that the virus particles of nuclear polyhedrosis of Amsacta albistriga Wlk. occurred singly or in bundles two to five rods while in H. zea and S. litura the virus rods were arranged singly and in bundles containing upto 14 rods. Livingston and Yearian (1972) observed single virion in the polyhedra or Pseudoplusia includens Walker and these polyhedra exhibited typical crystalline protein lattices. Smirnoff and Ackermann (1977) reported that the baculovirus of European skipper, Thymelicus

lineola Ochis., contained upto 80 single enveloped virions. Abnormally short and abnormally long particles were also observed. The bacilliform virions were enclosed in a polyhedral envelope. Polyhedra inclusion bodies containing rod shaped virions arranged in bundles in the protein matrix were described by Peter (1981) in Leucanobia fusca Den & Schiff.

2.3. Stage susceptibility

Differential susceptibility of lepidopterous larvae of different ages due to nuclear polyhedrosis virus was reported by Glaser (1928), Bergold (1943), Steinhaus (1949a), Bird (1953), Bird and Whalon (1953), Clark and Thompson (1954), Morris (1962a) Stairs (1965b), Doane (1967) Allen and Ignoffo (1969). Jacob and Subramaniam (1972b). Such increase in resistance to infection corresponding with the growth of the larvae was referred to as "maturation immunity" (Tanada, 1956), but Ignoffo, (1966b) attributed this partly to normal increase in body weight which in turn diluted a constant viral dose.

Nair and Jacob (1976) observed that in the case of the nuclear polyhedrosis of Pericallia ricini Fab. the caterpillars of second, third and fourth instars were highly susceptible. The incubation period was prolonged from 5.24 days in the second instar larvae to 8.20 days

in the fifth instar larvae. Mathew (1980) observed a prolongation of incubation period of NPV in the larvae of Anadevidia peponis Fab., from 2.15 days in the first instar to 4.40 days in the sixth instar larvae. The viral infection caused 100 per cent mortality of first, second, third and fourth instars. The fifth and sixth instar larvae showed less susceptibility, the mortality being 90 and 70 per cent respectively. Bergerjon et al., (1981) recorded an increasing resistance with age in the case of nuclear polyhedrosis of Lymantria dispar Linnaeus. Chan et al., (1981) reported that the infection of the first and second instar larvae was dose dependent and a concentration of $1.9 \times 10^{4-6}$ polyhedra killed 58-92 per cent of the larvae within 8 days of treatment.

Vail and Hall (1969b) studied the response of pupae and their resultant adults of the cabbage looper Trichoplusia ni Hubner to a nuclear polyhedrosis virus and found that diseased larvae seldom pupated or gave rise to moths. Pupae were susceptible to NPV administered injection. Moths emerging from pupae infected with virus were frequently deformed and many lacked the dark pigmentation of normal adults. Vail and Gough (1970) noted the infection of pupal tissues of T. ni also with the nuclear polyhedrosis virus.

Martignoni (1964) succeeded in infecting tissues of the adults of Peridroma saucia Hubner by inoculating polyhedrosis virus. Mathew (1980) recorded the pupal and adult mortality in Anadevidia peponis infected with nuclear polyhedrosis virus. Tissues of infected pupae were liquefied and the pupal cases were seen fragile. Infected adults were usually malformed, lacked the normal colouration and their tissues and body fluid contained millions of polyhedral inclusion bodies.

2.4. Histopathology of NPV infected insects

As the name implies the nuclear polyhedrosis viruses multiply in the nucleus of susceptible cells. Fat body, hypodermis, tracheal matrix, and blood cells were reported as the major sites of multiplication of nuclear polyhedrosis viruses (Aizawa, 1963; Smith, 1967; Okada, 1970; Injac et al., 1973; Rabindra and Subramaniam, 1974). Several other tissues such as silk glands, muscle cells, nerve sheath, brain ganglia, gonads, Malpighian tubules, connective tissues surrounding the midgut, epithelia of fore and hindguts, cardiac valve, pyloric valves and imaginal wing buds, have also been reported to be susceptible to the virus (Drake and McEwen, 1959; Tanada, 1960; Benz, 1963; Arugua et al., 1963; Stairs, 1965a, Adams and Wilcox 1968; Adams et al., 1968; Mathad et al., 1968;

Hamm, 1969; Vail and Hall, 1969a; Livingston and Yearian, 1972; Hunter et al. 1973; Jacob and Subramaniam, 1973).

Multiplication of nuclear polyhedrosis virus in the endodermal cells is rather unusual. Virus infection of midgut epithelial cells was observed in sawflies, Gilpinia hercyniae Hartig and Neodiprion sertifer Geoffroy. (Balch and Bird, 1944). Plusia chalcytes Esp (Laudoho and Anargior, 1963). T. ni (Heimpel and Adams, 1966) Ceramica picta Linnaeus (Adams et al., 1968) and Lythimna unipuncta Haw., (Tanada and Hess, 1970) and Pseudoplusia includens (Livingston and Yearian, 1972).

Infection of the midgut cells of the Eastern hemlock looper, Lambdina fiscellaria where a cycle of virus multiplication was completed without polyhedral formation was reported by Cunningham (1971). Pawar (1975) also observed virus multiplication in the midgut epithelium of Spodoptera litura without polyhedron formation.

Injac et al. (1973) reported the nuclear polyhedrosis virus of Hyphantria cunea Drury and they found them in the haemocytes. The disease was characterised by abnormal proliferation of hypodermal cells.

Jacob and Subramaniam (1973) observed hypertrophied nuclei with condensation of chromatin material in most hypodermal cells and some fat body cells of S. litura at 48 hours after inoculation. At 72 hours polyhedron formation was observed in the nuclei of the hypodermal cells. Nuclei at various stages of infection were observed in the fat body, blood cells, tracheae, muscles, ganglia, nerve sheath, silkglands and connective tissues. Infection appeared highly advanced with the rupturing of the nuclei in hypodermis, tracheal matrix, blood cells and fat body at 120 hours.

In Dasychira mendosa Linnaeus fat body, hypodermis, nerve and blood cells were the major susceptible tissues although polyhedra were also present in various connective and muscle tissues (Rabindra and Subramaniam 1975a). In Plusia peponis Fab., virus development was observed in the fat body and hypodermis at 72 hours after inoculation and in tracheal matrix, gut epithelium, sarcolemma of muscle tissues, neurilemma of nerve tissues and blood cells after 96 hours (Rabindra and Subramaniam 1975b).

Sugun et al. (1983) reported that though principally the nuclei of the fat body, hypodermis, hypodermal glands and tracheal matrix were the target tissues for replica-

tion of the polyhedrosis virus of Mythimna (Pseudaletia) separata Wlk., later the wing buds, imaginal discs, ovary, testes, silkgland, Malpighian tubules and sarcolemma revealed heavy infection. Finally polyhedral inclusion bodies were also observed in nuclei of neurilemma, frontal ganglion, thoracic ganglion and cerebral complex.

2.5. Mode of virus transmission to the progeny

Transovarial transmission was observed in Bombyx mori by Conte (1907) and Bolle (1908). Sagar (1960) observed virus like bodies in the eggs of seven species of Lepidopera. Although transmission of the virus through egg was generally accepted, the precise relationship of the virus to the egg was a matter of dispute (Bergold, 1953; Clark, 1955; Aizawa, 1963).

Bird (1961) reported transovum transmission of nuclear polyhedrosis virus in the sawflies, Diprion hercyniae Hartig Neodiprion sertifer and N. lecontei. Smirnoff (1961, 1962) observed virus transmission from adults through eggs in sawflies N. swainei, Trichiocampus irregularis Fallen and T. viminalis and once the virus had been introduced into the colony of N. swainei it

was maintained for years by transovum transmission. Martignoni and Milstead (1962) could get transovum transmission of nuclear polyhedrosis virus by application of virus paste to the external genitalia of adult female moths of the alfalfa caterpillar, Colias eurytheme Boisduval.

Elmore and Houland (1964) reported transovum transmission of the virus when cabbage looper moths were fed with polyhedra; no relationship was apparent between the mortality of the progeny and the number of polyhedra fed to the moths.

Harpaz and Ben-Shaked (1964) found that nuclear polyhedrosis virus of Prodenia litura was transmitted through egg and that it involved a complicated genetic mechanism. Brown and Swaine (1965) and Swaine (1966) observed that the larvae obtained from field collected females of L. exempta developed the disease even after adopting sanitary measures.

Doane (1969) demonstrated that transmission of the virus in gypsy moth Prothetria dispar took place by surface contamination of eggs. About 80 per cent of the larvae hatching from eggs of contaminated moths died of nuclear polyhedrosis in the first instar; the hatching

larvae getting the infection by ingesting part of the egg shells. Surface disinfection of eggs with 0.1 per cent sodium hypochlorite reduced the incidence of polyhedrosis in the susceptible larvae from 80.0 to 0.1 per cent.

Vail and Hall (1969b) reported that large number of progenies reared from cabbage looper moths which were infected with the virus rarely showed infection. In contrast, progeny of moth fed with the polyhedra were often infected, though the effect was unpredictable and could be eliminated by surface sterilization of eggs.

Pawar and Ramakrishnan (1971) found that in P. litura only a portion of the progeny of the infected female was diseased and the disease incidence was less in the eggs laid on the first day than those laid on the fourth day.

Hamm and Young (1974) reported that the nuclear polyhedrosis of H. zea was transmitted by surface contamination of eggs with inclusion bodies which had passed through the digestive tract of adults fed on polyhedra. Scanning electron micrographs showed polyhedra on the surface of eggs obtained from treated moths. They also found that males fed on polyhedra transmitted

the virus to the progeny when they paired with untreated females. It was also reported that the virus could pass from one female to another through the males. Similar observations were also made by Larionov and Dakhalov (1974) on the transovarial transmission of nuclear polyhedrosis of Lymantria monacha.

Nordin (1976) could demonstrate transovum transmission of the nuclear polyhedrosis of fall web worm, Hyphantria cunea by contaminating the adults with viral dust. Heslgund and Hetnad (1978) found that the nuclear polyhedrosis was transmitted by both transovum and transovarial routes in Mythimna separata. Kanai (1982) could demonstrate transovum transmission of a nuclear polyhedrosis virus of Hyphantria cunea in Japan.

2.6. Cross-infectivity

Ignoffo (1965b) reported that six species of Heliothis were susceptible to one isolate of nuclear polyhedrosis virus and the virus isolated from Heliothis peltigera was cross transmissible to three other Heliothis spp. A polyhedrosis virus isolated from Ephestia (Cadra) cautella Wlk., was found to be cross

transmissible to Plodia interpunctella Hubner (Hunter et al., 1973).

Rabindra and Subramaniam (1975b) reported that nuclear polyhedrosis virus isolated from Plusia peponis was cross infective to Trichoplusia ni. Rabindra and Subramaniam (1976) noticed the infectivity of the NPV of Plusia chalcytes to P. peponis. Considerable cross-infectivity of nuclear polyhedrosis virus was seen within the sub family Plusinae (Harper, 1976). Harpaz and Raccah (1978) found that the NPV of Spodoptera littoralis Boisduval could be cross transmitted to S. exigua and S. litura.

Some cases of interfamily and interordinal cross-infection have also been reported. Aruga (1962) found that the larvae of Galleria mellonella Linnaeus (Galleridae) were susceptible to nuclear polyhedrosis virus from silkworm Bombyx mori (Bombycidae). Larvae of Nemerobius sp. (Neuroptera) were found susceptible to nuclear polyhedrosis virus of Porthetria dispar (Lepidoptera) (Smith et al., 1959; Sidor, 1960). A nuclear polyhedrosis of Lymantria dispar sub sp. japonica (Motsch) was not infective to B. mori but was infective to Euproctis similis Fuessly, Dendrolimus

spectabilis Btlr., and Pygaeria anastomosis tritis Wlk. (Aratake and Kayamura, 1972) Vail et al., (1972) found that nuclear polyhedrosis virus isolated from alfalfa looper, Autographa californica Speyer was infective to Trichoplusia ni, Plutella xylostella Curtis Spodoptera exigua, Heliothis zea, Estigmene acrea Drury and Bucculatrix thurberiella Linnaeus. Aratake and Kayamura (1973) could transmit the NPV of B. mori to Dendrolimus spectablis, Malacosoma neustria testacea Linnaeus and Samia (Philosomia) cynthia preveri Butlerby oral administration and Chilo suppressalis Wlk., Galleria mellonella and D. spectabilis by injection into the haemolymph. Hyphantria cunea was found to be highly susceptible to the NPV of Thymelicus lineola (Brirnoff, 1976). Kaya (1977) reported the cross transmission of the NPV of A. californica to forest defoliators including Alsophila pometaria Harris and H. cunea. Lewis et al. (1977) could transmit the NPV of A. Californica to European corn borer, Ostrinia nubilalis Hubner. A nuclear polyhedrosis virus of Mamestra brassicae Linnaeus was found infective to Diparopsis watersi (Jacquemard, 1978). Stairs et al., (1981) reported the cross transmission of the NPV of Choristoneura fumiferana Clemens to Trichoplusia ni and Galleria mellonella. Heliothis

zea, T. ni and Sphingid Manduca sextra Joh., were found to be highly susceptible to the NPV of G. mellonella (Fraser and Stairs, 1982).

2.7. Pest control with nuclear polyhedrosis virus

2.7.1. Bio-assay of viral activity

Bio-assay technique has been widely used to evaluate the pathogenicity of virus preparations. Bergold (1953) reported the LD_{50} of 1.2 million polyhedra to the larvae of Bombyx mori and 5.5 million to the larvae of gypsy moth, Porthetria dispar and tent caterpillar, Malacosoma disstria Hubner. Bird and Whalen (1953) estimated the LD_{50} to the larvae of Neodiprion sertifer as 100 to 500 polyhedra. Morris (1962a) estimated an overall average median lethal dose (all instars) of 3000 polyhedra per larva of the western Oak looper, Lambdina fiscellaria somniaria. Ignoffo (1965a) found the LD_{50} of the NPV to 3 to 4 days old larvae of H. zea as 32 PIBs/mm^2 of diet surface. The LD_{50} values for first, second and third instar larvae of Porthetria dispar were 0.23, 2.3 and 2.5 PIBs/mm^2 of food surface respectively (Doane, 1967). An inverse relationship

between virus concentration and LT_{50} values were also observed for the first, second and third instar larvae. Nagnoler (1974) computed the LD_{50} of the NPV to third instar larvae of Porthetria dispar as 1729 PIBs/larvae or 729 PIBs/mg larval body weight. The LT_{50} for 2.5×10^6, 2.5×10^5, 2.5×10^4, 2.5×10^3 and 2.5×10^2 PIBs/larva were 8.1, 9.9, 11.3, 12.2 and 13.1 days respectively.

Hwang and Ding (1975) reported the LC_{50} for the third instar larvae of Prodenia litura as $1 \times 10^{5.3}$ PIBs/ml. Nagnoler (1975) estimated LC_{50} values of NPV to third and fourth instar larvae of Malacosoma neustria as 1405 and 12320 PIBs/larva respectively. The LT_{50} values for the concentration 3×10^5, 3×10^4 and 3×10^3 PIBs/ml were 5.9, 6.8 and 8.15 days respectively for the third instar larvae and 9.3 and 10.7 days for the higher concentrations in the case of fifth instar larvae. Pawar and Ramakrishnan (1977) estimated the LC_{50} values of NPV for the newly hatched and five day old larvae of Spodoptera litura as 0.089×10^6 and 7.79×10^6 PIBs/ml respectively. The LT_{50} values for the concentrations ranged from 5.41 to 9.95 days.

The dosage mortality study on the nuclear polyhedrosis virus of Spodoptera litura were carried out by Legaclon and Cabrlel (1978) and showed that 17.5×10^6, 12.1×10^7 and 90.05×10^8 PIBs/ 50 ml suspension gave 100 per cent mortality of the first instar larvae after an average of 11, 12.5 and 13.5 days respectively. The LT_{50} for the fifth instar larvae with the above doses were 11, 13.21 and 12.81 days. The LD_{50} values of the NPV to A. californica for cabra caterpillar, Cerconica pieta were found to be 63 PIBs/mm^2 and 3000 PIBs/mm^2 on diet surface for the first and third instar respectively (Espinera and Canoset, 1979).

Evans (1981) reported a 3400 fold increase in LD_{50} of NPV from the first to fifth instar larvae of Mamestra brassicae while the late fifth and sixth instar larvae were virtually resistant to virus infection. Blanco (1981) estimated the LC_{50} of third instar of Spodoptera exempta as 48.4 PIBs/larva and LT_{50} varied from 140.2 and 221.3 hours depending upon dosage.

2.7.2. Field efficiency of NPV

The forest pests controlled by the use of nuclear polyhedrosis viruses include pine sawfly, Neodiprion

sertifer (Bird, 1953), Great basin tent caterpillar, Malacosoma fragile Stretch. (Clark and Thompson, 1954), Wattle bagworm, Kotochalia junodi Heylaerts (Ossowski, 1960), Spruce sawfly, Diprion harcyniae (Bird and Burk, 1961) jack pine sawfly, Diprion swainai (Smirnoff, 1961; Cunningham and Entwistle, 1981), Spruce bud worm Choristoneura fumiferana (Stairs and Bird, 1962) and Gypsy moth, Portnetria dispar (Rollinson et al., 1965, Lewis, 1981).

Many experiments were conducted on the control of cabbage looper, T. ni by means of nuclear polyhedrosis virus. Hall (1957) obtained 100 per cent kill by a single application of 10 million polyhedra per ml while McEwen and Harvey (1958) found that 0.94 LE per acre was sufficient to initiate an epizootic. Hoffmeister and Litman (1961) reported the control of cabbage looper by weekly spraying of NPV at the rate of 5 LE/ acre while Getzin (1962) recommended weekly application of 9.5×10^9 PIBs/acre. Ignoffo et al., (1965) reported successful control of batt worm H. zea and tobacco bud worm H. virescens on cotton by the application of Baculovirus heliothis.

The NPV of Heliothis was applied on cotton at the rate of 10 to 100 LE per acre and it had shown increasing yield (McGarr and Ignoffo, 1966). Results of the field experiment by Allen et al., (1967) showed that five concentrations of NPV 1.5×10^{11}, 3×10^{11}, 6×10^{11}, 12×10^{11} and 18×10^{11} PIBs/acre compared favourably with insecticides in controlling these pests. Other workers also reported similar results (Kinzer et al., 1976) though in some cases only marginal control was obtained (Falcon et al., 1965; McGarr, 1968). Chapman and Bell (1967) reported that the virus 6×10^{11} to 6×10^{12} PIBs/acre was as good as or better than the insecticidal treatments.

Oatman et al., (1970) reported that the NPV of Heliothis, 228 LE per acre caused 77 per cent marketable ears. Creighton et al., (1971) tested H. zea NPV for controlling the tomato fruit borer and found that fruit damage by H. zea was 37 and 47 per cent for the two NPV formulations compared to 61 per cent damage in control.

Vail et al., (1972) also reported that in field plots of lettuce treated with NPV suspension of T. ni and A. californica gave adequate control of cabbage

looper and beet armyworm. Five applications of NPV at 1.8×10^{11} PIBs/acre suppressed the larval population of cabbage looper and the crop protection achieved, indicated that this virus was as effective as commercial insecticides (Jaques, 1975).

Jaques (1977) found the efficacy of the NPV of cabbage looper at 7.5×10^{11} PIBs/ ha for controlling the cabbage looper and the control obtained was comparable or better than that by Bacillus thuringiensis or chemical insecticides.

Hostetter et al., (1979) and Vail et al., (1980) also found that the virus of A. californica to be potentially useful pathogen for the field control of T. ni. The control obtained was comparable with that of chemical insecticides and Bacillus thuringiensis.

The results obtained by Santharam et al., (1980) indicated that a field dose of 100 LE of NPV of S.litura was sufficient to check the pest population on cotton appreciably. Increase in mortality of larvae was more when the virus was sprayed along with molases. The S. litura virus at 7.5×10^{11} PIB and 15×10^{11} PIB equivalent to 125 and 250 LE per ha and diflubenzuron

0.01 per cent gave only less than 30 per cent control 48 hours after application. But after one week all the treatments gave good control of S. litura on tobacco (Santharem and Balasubramanian, 1980).

2.7.3. Persistence on foliage

Continuous exposure at high field temperature was found to affect viral stability and inhibit viral multiplication. (Bird, 1955; Thompson, 1959; Ignoffo, 1966b). According to Smith (1967) inclusion viruses could persist longer than non-inclusion viruses because the protinaceous crystal protected the virus from the effect of unfavourable environment. Ignoffo (1968) observed that the environmental factors such as light, temperature, rainfall, free water, pH and plant interaction might have deactivated field applied viruses. But normal range of physical components of the environment were not found to be destructive to most viruses. Laboratory tests indicated that normal field temperature (10-30°C) did not adversely inhibit viral activity or viral stability in diseased caterpillars.

The inactivation of Baculovirus heliothis under field conditions was attributed to high temperature

(Gudauskas and Canerday, 1968), solar irradiation particularly in the UV spectrum (Bullock et al., 1970)

Cunningham (1970) found that the nuclear polyhedrosis virus of eastern hemlock looper, Lambdina fiscellaria fiscellaria on balsom foliage remained fully effective for three days after spraying, declined in pathogenicity from the fifth day and was completely inactivated by tenth day.

Okada (1972) observed that the viral activity of the nuclear polyhedrosis of S. litura decreased rapidly after spraying under outdoor conditions, but much less in a glasshouse or darkroom. Under outdoor conditions the polyhedra applied in the spray with gelatin retained their activity than those applied without gelatin suggesting that the thickness of gelatin coat on the polyhedra was a factor in preserving viral activity.

Andrews and Sikorowski (1973) reported that persistence of nuclear polyhedrosis virus of H. zea was lost at night. Dew from cotton foliage was shown to contain various alkaline substances and the virus, although not affected by a pH of 9, but 99 per cent

inactivated at pH 12. Such inactivation of Baculovirus of Heliothis under field conditions was attributed to high pH of dew on cotton foliage (Andrews and Sikorowski, 1973; Mcloed et al., 1977). Ignoffo et al., (1974) found the half life of NPV of Heliothis on soybean foliage to be between two and three days. Persistence of the virus was increased by the use of protectants and by caging the plants during day time.

Young and Yearian (1974) studied the persistance of Heliothis nuclear polyhedrosis virus on foliage of cotton, soybean and tomato and found that the inactivation was most rapid on cotton with little activity remaining after 24 hours. Persistence was significantly better on tomato than on other plants.

Rooms and Daoust (1976) found that the activity of NPV of H. armigera persisted more than 80 days after spraying. Entwistle and Adams (1977) observed prolonged retention of infectivity of the NPV of Gilpinia hercyniae on the foliage of spruce species.

2.7.4. Effect of sunlight and ultra violet light on the virulence of nuclear polyhedrosis viruses

Ultraviolet light was reported to deactivate viruses under laboratory conditions (Watanabe, 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 the NPV of Heliothis applied on cotton foliage lost most of its viral activity in one day and he attributed UV light to be a reason for this loss. Cantwell (1967) found that the NPV of T. ni and Antigmena acrea were inactivated when exposed to direct sunlight for three house.

Jaques (1967a) showed that deposits of polyhedra of cabbage looper virus on cotton foliage was practically non-infective one month after exposure to weathering and sunlight was attributed to this inactivation. A case of photo-reactivation of a UV inactivated granulosis virus of Pieris rapae Linnaeus when exposed to direct sunlight immediately after exposure to the former was

reported by David and Magnus (1967).

Studies on the NPV of Lambdina fiscellaria somniaria by Morris (1971) showed that UV radiation of predominantly 3600 Å wavelength was slightly viricidal and that gamma radiation caused no substantial reduction in pathogenicity of the virus. But exposure to sunlight beyond five hours decreased the pathogenicity of the virus and only eleven per cent pathogenicity persisted after 35 hours.

Okada (1972) found that the viral activity of inclusion bodies of the NPV of S. litura decreased rapidly after spraying under outdoor conditions but much less in a glass house or dark room. The NPV of Malacosoma disstria was inactivated within 10 hours of exposure to sunlight (Broome et al., 1974). Lathika and Jacob (1974 c) reported that the NPV of S. mauritia as thin films of PIBs on glass surface lost most of its infectivity after 96 hours of exposure to sunlight. Okada (1974) found that egg albumen, egg yolk or artificial diet and excreta of the larvae were as effective or better than gelatin in protecting the nuclear polyhedrosis virus of S. litura from inactivation by sunlight.

Elgee (1975) suggested that the rate of inactivation depended on the degree of exposure to weathering and the persistence of the virus depended on this and the concentration of the suspension. Ramoska et al., (1975) observed an activation of virus of A. californica by exposure to ultraviolet rays of 320 nm for 60-420 seconds. Jaques (1977) reported that the nuclear polyhedrosis and granulosis viruses were rapidly inactivated by exposure to sunlight, strong acids, or alkalies and high temperatures. Inactivation by sunlight was apparently the most important factor causing loss of activity of viruses in the field.

Narayanan et al., (1977) found that NPV of S. litura could not withstand prolonged day light and heat under field conditions and suggested that repeated application of the pathogen might be necessary for effective control of the pest.

The NPV of Mythimna separata was found to survive normal field conditions as regards temperature and ultraviolet light (Manjunath and Mathad, 1978). Pawar and Ramakrishnan (1979) obtained complete inactivation of the NPV of S. litura in 15 minutes when exposed to UV

light and considerable loss in infectivity by eight hours of exposure to sunlight.

Timans (1982) reported that the NPV of gypsy moth, Lymantria dispar was highly sensitive to small doses of UV light, 96 per cent inactivation being observed when exposed for two minutes at 230-320 nm. Further examination under the particular wavelength, using UV filters, showed that short wave length of both artificial light and sunlight had the most pronounced inactivation effect on the virus.

2.8. Heat Inactivation of the virus

The thermal inactivation of the inclusions and enclosed viruses was different from the thermal inactivation of most proteins in laboratory testing (Aizawa, 1953; Bergold, 1953) . Several workers reported that higher field temperature affected viral stability and inhibited viral multiplication (Bird, 1955; Thompson, 1959).

Morris (1971) reported that heating the NPV of Lambdina fiscellaria somniaria at 45°C for 200 hours did not affect the final percentage of mortality.

Broome et al., (1974) reported that the ten hour exposure to sunlight caused total loss of activity of aqueous suspension of NPV of Malacosoma diastria.

Nair and Jacob (1976) found that the NPV of P. ricini was highly ineffective even after 96 hours of exposure to a constant temperature of 35°C. Martignoni and Iwai (1977 a) studied the thermal inactivation of two strains of the NPV of Orgyia pseudotsugata and found that they were inactivated by a first order of reaction at 55°C and 60°C. The SV virulent strain lost its infectivity completely when exposed to 60°C for 240 minutes while the HV strain required 1320 minutes for complete inactivation at 60°C. Stairs (1978) reported the complete cessation of development of NPV of Galleria mellonella at 40°C. Pawar and Ramakrishnan (1979) observed a gradual decline in the infectivity of S. litura NPV with prolonged exposure to 40 and 50°C.

Stairs and Milligen (1980) reported that the 50 per cent of inactivation of NPV of G. mellonella was obtained with exposure to 42°C for 329 minutes.

The purified virus had a higher initial virulence than crude virus and the latter persisted longer in the field (Elnagar and Abdul Nasar, 1980). Boucias et al. (1980) observed that the NPV of velvet bean caterpillar, Anticarsia gemmatilis Hubner replicated effectively at 26.7°C but the incubation at higher temperature of 32.2°C caused 30-50 per cent mortality than at lower temperature.

2.9. Safety of nuclear polyhedrosis virus

2.9.1. Safety to parasites and predators

Beegle and Oatman (1975) studied the effect of nuclear polyhedrosis virus on T ni and its parasite Hyposoter exigua Vier. No difference in the fecundity of parasites that developed on virus infected hosts and those developed on uninfected host was noticed. Female parasites laid as many eggs in virus infected host as they did in uninfected hosts.

Hassan and Groner (1977) observed that when adults of Trichogramma cacoeciae Marchal were exposed to a fresh day deposit of an aqueous suspension of the NPV of Mamestra brassicae L., there was no reduction in their parasitising

ability. It was also shown that the deposit of viral suspension had no repellant effect on the parasite. There was no adverse effect on the larval feeding capacity, adult female fecundity or egg viability of Chrysopa carnea Steph.

Raimo et al., (1977) reported that the larvae of L. dispar reared on artificial diet when exposed to parasitism by females of Apanteles melanoscelus Ratz., which had been contaminated with the NPV showed no significant difference in emergence.

Tests on pathogenicity of the NPV of H. armigera against emerged adults of four indigenous and eight exotic parasitoids, two species of Chrysopa (both adult and larvae) two species of Coccinellid predators, one species of predatory mite revealed that NPV was safe to all parasites and predators tested (Narayanan, 1980).

2.9.2. Safety to productive insects

Dhaduti and Mathad (1980) observed that the NPV of Mythimna separata Wlk. caused no significant harm to colonies of Apis cerana indica F. Narayanan (1980) reported that the NPV of H. armigera was safe to two species of silkworm.

2.9.3. Safety to birds and fishes

Ignoffo (1969) conducted feeding tests of NPV of *H. zea* to English sparrow, Valley quail and Mallard duck and found that the NPV was safe to the test organisms. Narayanan et al., (1977a) reported that the NPV of *Amsacta albistriga* Wlk., was harmless to common carp, *Cyprinus carpio* Lin., Narayanan et al., (1978) observed that poultry birds when fed with the nuclear polyhedrosis virus of *A. albistriga* and a single heavy dose of 0.1 ml of the virus suspension containing 16.48×10^9 PIBs/bird did not show any dearrangement in their general condition, internal organs and biochemical content of blood.

Detailed studies were conducted using the NPV of *H. armigera* against two species of fish and poultry birds by Narayanan (1979). No deleterious effect, directly attributable to the virus was observed.

Lautenschlager et al., (1980) studied the natural occurrence of the NPV of the Gypsy moth, *L.dispar* in wild birds. Infectious polyhedra were found in the gut content of blue jays (*Cyanocitta cristata* Lin.), Towhees (*Pipilo erythrophthalmus* Jerd.), Red backed Voles (*Clethrionomys gapperi* Linn.) But he observed that the birds served only as carriers of the virus.

2.9.4. Safety to mammals

Ignoffo and Heimpel (1965) tested the NPV of H. zea on white mice and guinea pig. Mice and guinea pig were exposed to virus powder (500 g/mouse and 100 g/guinea pig) in closed containers for two hours and the animals were observed for two months. None of them became unhealthy and all the test animals made normal weight gain over the test period. Heimpel (1966) carried out a similar test with the NPV of T. ni and found that there was no evidence of toxicity or pathogenicity to the animals tested. Lautenschlager and Podgwaite (1977) found that White footed mouse (Peromyscus leucopus, Lin) and short tailed shrew (Blarina brevicauda, Lin.) and two small mammal predators of Lymantria dispar Motsch., passed significant amount of the nuclear polyhedrosis virus through their alimentary tract, resulting in the distribution of NPV in the natural environment.

Narayanan et al., (1977b) exposed White mice to Baculovirus amsacata and found that the animals were healthy and there was no change in their bodily appearence, behaviour and feeding activity. Histological examination of heart, lungs, liver, spleen, testes and

ovary showed no evidence of tissue damage and they were similar to the corresponding tissues of the healthy animals.

Lautenschlager et al., (1977) reported that no adverse effect of NPV could be detected in white footed mice (Peromyscus leucopus) Lin.) short tailed shrew , Blarina brevicauda) and Didelphio virginiana Lin., when they were fed with NPV infected larvae or provided with PIBs mixed in dog food or when applied in a standard spray formulation.

Detailed studies were conducted using the NPV of H. armigera against white rats by Narayanan (1979). He found no deleterious effect directly attributable to the NPV of H. armigera on test animals when the virus was administered orally.

Lautenschlager et al., (1980) studied the natural occurrence of NPV of the gypsy moth L. dispar L. in mammals. Infectious polyhedra were found in the internal content of white footed mice (Peromyscus leucopus), raccon (Procyon lotor Lin.), and chip munta (Tamias striatus Hume.)

It was noted that the mammals acted as passive carriers of the virus and aided its dispersal.

Doller and Groner (1981) reported that the NPV of Mamestra brassicae L. did not replicate in vertebrates and there was no risk to vertebrates in the use of the virus for the biological control.

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Mass culturing of Opisina arenosella

Materials used for rearing the insects (troughs, bell jars and plastic containers) were sterilized by keeping them immersed in 0.5 per cent sodium hypochlorite solution for one day (Wittig, 1963). They were then washed in distilled water and air dried. The smaller glasswares and tubes were sterilized by keeping them in a hot air oven at 180°C for three hours.

The initial culture was built up with eggs of O. arenosella collected from field. The eggs were surface sterilized by immersing the leaf bits containing the eggs in 10 per cent formalin for one hour (Henneberry and Kishaba, 1966). They were then washed in several changes of sterile water and the moisture was then removed by air drying. These eggs were kept in clean sterilized glass trough for hatching. A bouquet of tender leaves of coconut, with the basal portion covered with wet cotton swab to maintain turgidity, was provided in the trough as food for the emerging larvae (Fig.1). The top of the trough was covered with muslin cloth. On the third day of emergence the

larvae were transferred in batches of 25 to 30 into sterilized glass troughs containing fresh coconut leaves. The leaves were then changed once in a week. Larvae showing symptoms of diseases were promptly removed from the culture. The larvae generally pupated on the leaves within the silken galleries and the adults emerged in 7 to 10 days.

The adults were kept in glass battery jars for egg laying (Fig.2) in batches of five pairs. Ten per cent honey in cotton swab, was provided as food for the moths. Egg laying commenced one to two days after emergence and it continued for three to four days. Fresh leaves were provided daily in the jars to serve as substrate for egg laying. Leaf bits bearing the eggs were cut out and the eggs were sterilized and used for further rearing as described earlier.

3.2. Multiplication of virus inoculum

The primary inoculum was obtained from a dead larva of O. arenosella collected from field and polyhedral suspension was prepared in distilled water. It was fed to the early instars of O. arenosella in the laboratory by contaminating the coconut leaves used for feeding them. The polyhedra were collected

Fig. 1. A bouquet of coconut leaf exposed for egg laying.

Fig. 2. Opisina arenosella adults exposed to coconut leaves in glass jars for egg laying.

FIG. 1

from infected larvae following the method described by Smith (1967). The dead larvae were allowed to putrify in distilled water in large conical flasks for two to three weeks at room temperature. 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 centrifugation. The purified polyhedra were suspended in sterile distilled water and stored in a refrigerator at 4°C.

3.2.1. Preparation of a stock suspension of the polyhedra

The concentration of polyhedra in the stock suspension prepared was estimated by a Neubauer double ruled haemocytometer following the method of Lewis (1960). A stock containing 1.96×10^7 PIBs/ml was prepared by adding required quantity of sterile water and the same was used as a stock suspension in all the experiments.

3.3. Selection of test larvae

Symptomatology and incubation period of the virus were studied in all larval instars. For the other

experiments third instar larvae collected within six to eight hours after the second moult alone were used. But for the transmission studies late fourth instar larvae were used.

3.4. Inoculation of caterpillars with the virus

The spot feeding technique developed by Jacob (1972) was adopted for all the inoculations. Pieces of middle aged coconut leaflets were fixed on a thick cardboard with pins, the undersurface of the leaf facing down. Pieces of paper gum tapes, one inch square, with a circular hole of 6 mm diameter punched in the middle were pasted over the exposed surface. With a micropipette, 0.05 ml of the polyhedral suspension containing 0.1 per cent teepol as wetting agent was placed on each of the circular exposed leaf discs and the suspension was allowed to dry at room temperature. One larva each was confined to each inoculated spot by using an inverted penicillin vial (Fig.3). Larvae similarly fed with 0.05 ml of 0.1 per cent teepol in water served as control. The larvae which had completely ingested the treated leaf disc in 8 to 12 hours were transferred to glass troughs in batches and were supplied with virus free coconut leaf.

Fig. 3 Spot feeding technique for inoculating NPV to the larvae of Oscinia arenosella

FIG 3

3.5. Diagnosis of the disease

The larvae dying with viral infection were diagnosed by microscopic examination of squashed preparations of tissues for the presence of polyhedra.

3.6. Electron micrography

The electron micrography of the virus was got done by NERC Institute of Virology, Oxford, England.

3.7. Histopathology

Third instar larvae of *O. arenosella* were inoculated with a viral suspension of 1.96×10^7 PIBs/ml as described earlier. At 24 hour intervals and upto 144 hours after inoculation, three larvae each from the inoculated and control groups were collected at random and used for histopathological studies. A similar set of larvae fed on 0.1 per cent teepol alone served as control. The larvae were fixed in hot alcoholic Bouin's fixative. After ten minutes smaller specimens cut into two and larger ones into three pieces were transferred to fresh fixative and were kept at room temperature for 24 hours (Drake and McEwen, 1959). Then they were washed repeatedly in 70 per cent ethanol until the yellow colour was completely removed. The specimens were dehydrated in an ethyl alcohol-butyl alcohol series and embedded in

paraffin. Serial longitudinal and cross sections were taken at five microns and were given azan stain as recommended by Hamm (1966).

3.8. Assessment of the mode of virus transmission of the NPV to the progeny of the host

Two hundred, fourth instar larvae of O. arenosella were inoculated with polyhedral suspension containing 1.96×10^7 PIBs/ml by the spot feeding technique. Another set of two hundred larvae of the same age group fed similarly on coconut foliage treated with one per cent teepol in water served as control. Both lots were reared under aseptic conditions in glass troughs. The pupae obtained from both groups were kept separately in glass troughs. The moths on emergence, were grouped in batches of five pairs for egg laying as described earlier.

3.8.1. Assessment of transovum/transovarial transmission

One batch of eggs laid by moths obtained from virus treated larvae was surface sterilized by soaking them in ten per cent formalin for one hour. Another batch of eggs laid by the same groups of moths was left unsterilized. Four replications of 50 eggs each from the steri-

lized and unsterilized lots were kept separately for hatching and symptoms of infection in the emerging larvae were observed. Similar lots of eggs laid by moths obtained from untreated larvae were kept without surface sterilization as control. The larvae were reared in the laboratory on fresh food material and the larval mortality, pupation and adult emergence were recorded.

Another lot of eggs obtained from moths which developed from virus treated larvae were divided into two batches. The first batch of eggs was throughly homogenised in a mortar and pestle, filtered through a cheese cloth and the filtrate was made upto a known volume by adding sterile distilled water. The second batch of eggs was surface sterilized by immersion in ten per cent formalin for one hour and a homogenate was prepared as described above. The viral activity of the above homogenates mixed with teepol (0.1 per cent) was tested against third instar larvae of O. arenosella adopting the spot feeding technique. Another set of larvae fed on leaves smeared with distilled water containing 0.1 per cent teepol alone served as control. The larvae in each treatment were reared separately.

There were four replications of 25 larvae each for every treatment. The larval mortality, pupation and adult emergence were recorded.

3.8.2. Transmission through virus fed moths

Adults developed from disease free larvae reared under aseptic conditions were used in this experiment. Five pairs of adults were fed with a polyhedral suspension of 1.96×10^7 PIBs/ml in 10 per cent sucrose solution. Each pair was confined in a wide mouthed glass bottle. The mouth of the bottle was covered with a nylon net over which was placed a cellophane disc of 5 cm diameter with a 2 mm hole punched at its centre. A cotton swab soaked in the polyhedral suspension in sucrose solution was placed outside the cellophane disc and at the centre. The cotton swab was then covered with an inverted petridish to prevent drying. The bottle and petridish were again inverted and kept. The moths could thus be fed on the NPV mixed food avoiding contamination of the body with the virus. Moths fed similarly in 10 per cent sucrose solution alone served as control. The moths were then kept in the laboratory in pairs for egg laying. There were four replications for each treatment including control. Twenty five eggs collected at random from each

replication was surface sterilized in 10 per cent formalin for one hour and kept for hatching. Another set of 25 eggs collected from the same pair was kept without surface sterilization. Larvae developed from each replication were reared separately. Larval, pupal mortality and adult emergence were recorded.

3.8.3. Transmission through contamination of female genitalia

Ten gravid female moths reared from healthy larvae under aseptic conditions were selected. A polyhedral suspension of 1.96×10^7 PIBs/ml containing 0.1 per cent teepol was smeared on the abdominal tips of five female moths using a camel hair brush. Abdominal tips of the other five female moths were smeared with 0.1 per cent teepol alone and they served as control. The moths were then confined separately for egg laying. The eggs laid by the contaminated moths were divided into three batches of 50 each. One batch in each case was surface sterilized in 10 per cent formalin for one hour. The other batches of eggs in each was kept without surface sterilization and the emerging larvae were reared as above. An equal number of eggs obtained from contaminated moths was also kept for hatching and the larvae were reared separa-

tely. Larval and pupal mortality and adult emergence were recorded. There were three replications of fifty eggs each.

3.9. Assessing cross-infectivity of the virus to the larvae of different families of lepidopetera

Cross-infectivity of the nuclear polyhedrosis virus of O. arenosella to Spodoptera litura F. (Noctuidae), Pericallia ricini F. (Arctiidae) and Euproctis fraterna Moore. (Lymantridae) on castor, Spodoptera mauritia Bosid. (Noctuidae) and Cnaphalocrocis medinalis Guen. (Pyralidae) on paddy. Diacrisia obliqua Wlk. (Arctiidae) on sweet potato, Orthaga exvinacea H. (Pyralidae) on mango, Andadevidia peponis F (Noctuidae) and Margaronia indica Saund. (Pyralidae) on snakegourd and Sylepta derogata F. (Pyraustidae) on bahindi were studied.

The test larvae were fed with leaves of their respective host plants treated with a concentrated polyhedral suspension (1.96×10^7 PIBs/ml) containing 0.1 per cent teepol as wetting agent. After feeding on the treated leaves for one day the larvae were transferred to fresh uncontamined leaves and reared until pupation or death. There were twenty five larvae in each replication and three replication for each host. An equal number of larvae fed on leaves dipped in

0.1 per cent teepol alone was kept as control. Observations were made on larval mortality, pupal mortality and adult emergence. The larvae dying with viral infection were diagnosed by microscopic examination of the squashed preparation of the tissues for the presence of polyhedra. The concentration of polyhedra in the stock suspension was estimated by techniques described earlier. The purified polyhedra obtained from each host was suspended in sterile distilled water and fed to the laboratory reared third instar larvae of O.arenosella adopting spot feeding technique for assessing the pathogenicity of the pathogen obtained from different hosts.

3.10. Evaluating the efficacy of the virus against O. arenosella

3.10.1. Bioassay of the virus

Eight serial dilutions of the PIBs were prepared from the stock suspension and lowest six serial dilutions were used for the first instar larvae where as highest six serial dilutions were used for the other larval instars. The test larvae were drawn from the disease free laboratory stock of O. arenosella. All the five larval instars were used in the bioassay. Larvae of uniform age and size were used for each assay. Larvae were inoculated with

the virus by the spot feeding technique. After feeding the treated bits, fresh bits of coconut leaves were supplied to the larva confined individually in specimen tubes. The treatments were replicated four times with ten larvae in each replication. The larval mortality as well as pupation and adult emergence of surviving individuals were recorded. In doubtful cases, mortality due to nuclear polyhedrosis was confirmed by microscopic examination of smears from dead larvae.

The mortality data obtained were subjected to probit analysis (Finney, 1952). The LC_{50} of the virus for each larval instar was calculated. The LT_{50} values for the various doses of PIBs also were computed.

3.10.2. Persistence of the virus on coconut foliage

Eight year old coconut palms grown in open field were selected for the experiment. Eight hundred and forty, 6 mm diameter spots were marked on leaves which were within reach from ground. Leaves under shade were avoided for the purpose. Five micro litre of the virus suspension (1.96×10^7 PIBs/ml) containing 0.1 per cent teepol was placed over each marked spot on the leaves. Areas treated similarly with five microlitres of 0.1 per cent

teepol in water alone served as control. Thirty treated leaf spots each were cut out from the treatment and control, at intervals of 12 hours and upto the end of 168 hours after treatment. The viral activity was assayed by allowing one third instar larvae to feed on each spot adopting the technique described earlier. The leaves were maintained under room temperature which ranged from 28.5°C to 31.5°C and the relative humidity ranging from 70 to 80 per cent. Incubation period of the virus, larval mortality, pupation and adult emergence were recorded. The data obtained were subjected to statistical analysis and LT_{50} values were also computed.

3.11. Effect of continual exposure of the polyhedra to a constant temperature of 35°C on the viral activity

Dried films of polyhedra were prepared in thirty-nine petridishes and they were exposed to a constant temperature of 35° C in an incubator. A similar lot kept under room temperature in another incubator (disconnected from electric mains) served as control. Three dishes each were removed from both the incubators at 12 hour intervals upto 156 hours. These were cooled to room temperature and the NPV in each dish was then

suspended in two ml sterile distilled water containing 0.1 per cent teepol. The virus activity of these suspensions was assayed against third instar larvae of *C. arenosella* adopting spot feeding technique. Ten larvae were used for each replication. The larval mortality at different intervals after ingestion of the virus was recorded. The data were subjected to statistical analysis and the LT_{50} values were also computed.

3.12. Effect of Infra-red rays on viral activity

Air dried film of polyhedra prepared in thirtynine petridishes as in the previous experiments were exposed to infra red rays emitted from a Philips 150 w infra red lamp kept at a distance of 30 cm from the sample. A similar lot kept under room temperature without exposure to infra red rays served as control. At 12 hour intervals three petridishes were removed from the light source and from the control group. The PIBs in each sample was suspended in two ml sterile distilled water containing 0.1 per cent teepol. The viral activity of the suspension was assayed against third instar larvae of *C. arenosella* as in the previous experiment. The larvae were then kept at room temperature. The larval mortality

was recorded. The data obtained were subjected to statistical analysis and the LT_{50} values were computed.

3.13. Effect of ultraviolet light on viral activity

Two ml aliquots of a suspension containing 1.96×10^7 PIBs/ml were taken in clean sterilized petridishes and the suspension in each dish was allowed to dry as a thin film under an electric fan. The dried films of polyhedra were then exposed to UV rays emitted from a Philips UV-germicidal lamp kept at a distance of 30 cm from the dishes. Thirty nine dishes were thus set up. same number of dishes with virus film kept in the laboratory without exposure to UV light served as control. At 12 hour intervals three petridishes each were removed from the UV source and control. The NPV in each dish was suspended in two ml sterile distilled water containing 0.1 per cent teepol. The suspension from each dish was assayed by exposing ten, third instar larvae of *O. arenosella* adopting spot feeding technique. The larval mortality was recorded. The experiment was carried out under room temperature which ranged from 28.5°C to 30.5°C and relative humidity ranging from 85 to 90 per cent. The data obtained were subjected to statistical analysis and the LT_{50} values were computed.

3.14. Safety of the pathogen

3.14.1. Susceptibility of silkworm to NPV of O. arenosella

These studies were conducted on third instar larvae of Bombyx mori. Twenty larvae were inoculated with 0.05 ml of polyhedral inclusion bodies (1.96×10^9) as done in the case of O. arenosella. An equal number of larvae were allowed to feed on mulberry leaf discs treated with sterile distilled water containing 0.1 per cent teepol alone which served as control. When the leaf spot was eaten completely the larvae were reared in individual labelled containers. Fresh uncontaminated mulberry leaves were provided daily. There were four replications for each treatment. Larval mortality, pupation and adult emergence were recorded.

3.14.2. Susceptibility of Bracon brevicornis to NPV of O. arenosella

Adult of B. brevicornis reared under aseptic conditions were used in this experiment. Fifty early third instar larvae of O. arenosella were inoculated with polyhedral suspension of 1.96×10^7 PIBs/ml adopting the spot feeding technique. Another set of larvae of the same age group fed with virus free coconut leaves served as control. Inoculated and untreated larvae were reared

under aseptic condition. They were divided into five groups of 10 each. Ten adults of B. brevicornis were released into each tube. The adult mortality, general behaviour, and fecundity of the parasite were recorded. The emerged parasites were reared separately in labelled glass specimen tubes for recording the disease development if any.

In a second experiment ten pairs of adults of B. brevicornis were fed with a polyhedral suspension of 1.96×10^7 PIBs/ml in 10 per cent sucrose solution provided in cotton swab kept at the side of glass tube. The mouth of the tube was covered with muslin cloth. Adults fed similarly with 10 per cent sucrose solution alone served as control. Five replications were kept for the treatment and control. After feeding, each group was given third instar larvae of O. arenosella for oviposition. The general behaviour of the parasite and adult mortality were recorded. The tests were conducted at room temperature ranging from 25 to 28°C and relative humidity of 87 to 90 per cent.

3.14.3. Susceptibility of Trichospilus pupivora to NPV of O. arenosella

Fifty early fifth instar larvae of O. arenosella were

inoculated with polyhedral suspension of 1.96×10^2 PIBs/ml by the spot feeding technique described earlier. Another set of fifty larvae of the same age group fed with virus free coconut leaves served as control. Both inoculated and control larvae were reared in groups of ten each under aseptic condition in glass specimen tubes. The pupae obtained from both groups were kept separately. Twenty T. pupivora adults were released into each of the specimen tubes. The general behaviour of the parasite and emergence of adult parasitoids from the pupae were recorded. T. pupivora developed from each group of pupae were maintained separately for recording disease development if any.

3.14.4. Effect of the NPV of O. arenosella on white mice and white rats

Two to four week old randomly mated non inbred lines of white mice (average weight 25.990 g, range 25.000 to 26.900 g) were used in the study. A highly purified virus suspension containing 21.44×10^8 PIBs/ml was orally administered @ 0.2 ml/animal. Ten animals receiving only 0.2 ml of sterile distilled water alone were used as control. The animals were maintained in separate cages in groups of five each. Each treatment and control were replicated five times. Standard food and adequate

quantity of water was supplied ad libitum.

The general appearance and behaviour of the white mice were recorded daily and the body weight and temperature were recorded at weekly intervals for 21 days.

In a second experiment 25 numbers of four to seven week old randomly mated non inbred lines of white rats (average weight 108.20 g; range 107.40 to 109.00 g) were chosen and purified virus suspension containing 21.44×10^8 PIBs/ml was orally administered @ 0.2 ml per animal. Ten white rats receiving 0.2 ml each of sterile distilled water served as control. The animals were maintained in separate cages in groups of five each and each treatment was replicated five times while the control had two replications only. Standard food and water were supplied ad libitum. The animals were examined daily for general appearance and behaviour for 21 days. Body weight and temperature were recorded at weekly intervals.

At the end of the test period of 21 days, the test animals were etherised and blood was withdrawn by cardiac puncture from two animals in each replication. Potassium oxalate was used to prevent coagulation.

The blood counts were conducted following the standard procedures.

Then the animals were dissected and wet weight of the important organs viz. heart, liver, lungs, spleen, kidney, testes and ovary were recorded. Gross pathological and histopathological observations on the above organs were also made.

3.14.5. Effect of NPV of O. arenosella on developing chick embryo

3.14.5.1. Selection of eggs

Hundred numbers of embryonated chick eggs (7 to 9 days old) were used in the study. Care was taken to select only naturally clean, medium sized, white shelled egg with a fertility rate of above 95 per cent. This ensured embryos of normal dimension. All the eggs were incubated in the horizontal position. During the entire preinoculation incubation period they were turned twice daily through 180^{o} in order to assist symmetrical embryonic development and prevent adhesion of the embryonic membranes.

3.14.5.2. Candling

The progress of embryonic development was followed by candling the eggs daily during incubation period.

Candling consisted of viewing the egg against a concentrated light source, so that the shadow of the embryo and its associated structures were visible.

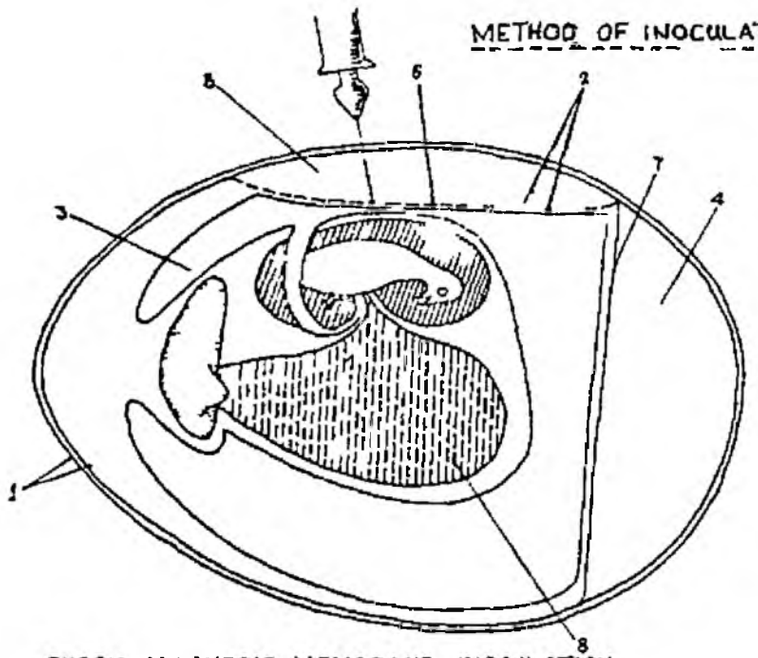
3.14.5.3. Inoculation

NPV was introduced into the Chorio allantoic membrane, allontoic cavity, amniotic cavity and yolk sac. (Fig.4). Each treatment was replicated five times with 20 eggs in each replication. Egg shells were surface sterilized by a swab lightly squeezed in tincture of iodine. Care was taken to avoid too much soaking of the shell with the solution. They were inoculated with 0.2 ml of highly purified viral suspension (21.44 x 10^8 PIBs/ml). Twenty eggs receiving 0.2 ml of sterile normal saline served as control. The inoculations of viral suspension were conducted following the standard embryonated egg inoculation technique of Hoskins (1967).

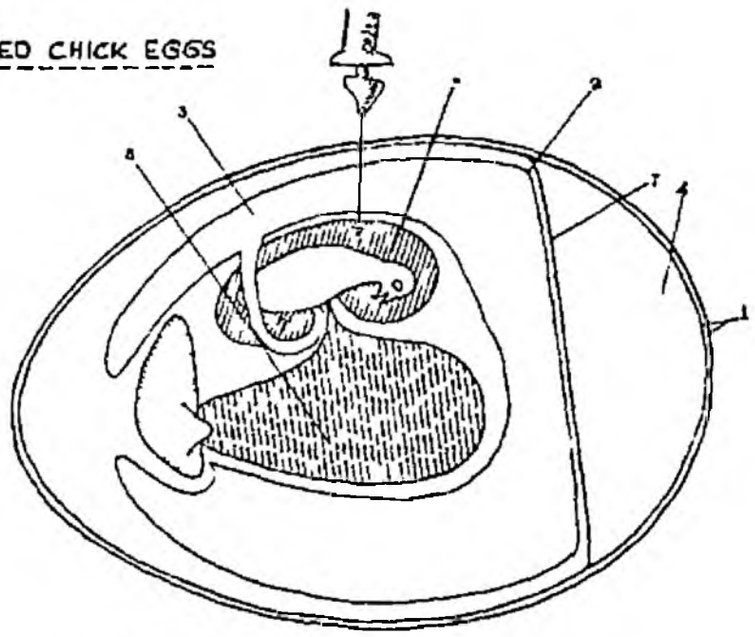
The general condition of the embryo (dead/alive) was observed daily and at the end of the test period the embryos were killed by placing the eggs in deep freezer and chorio-allantoic membrane, allantoic fluid, amniotic fluid and yolk were examined for the presence of polyhedra.

Fig.4 Method of inoculation of embryonated chick eggs.

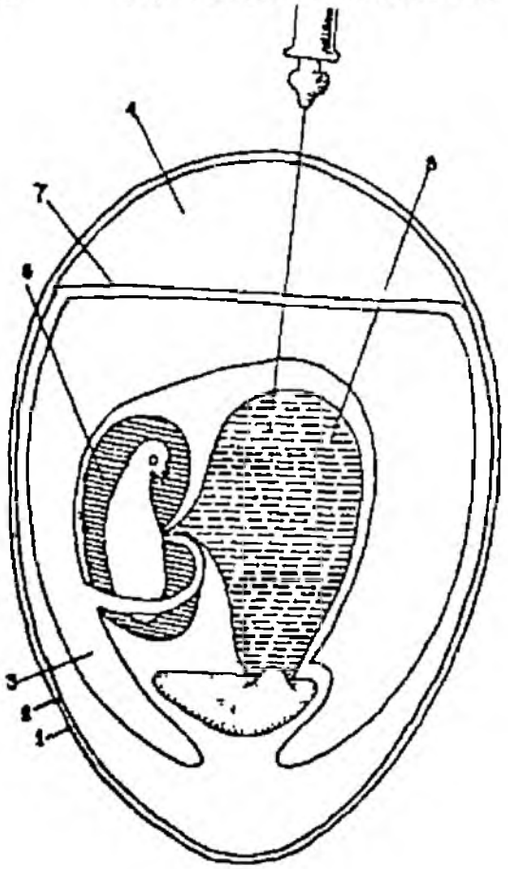
METHOD OF INOCULATION EMBRYONATED CHICK EGGS



CHORIO ALLANTOIC MEMBRANE INOCULATION

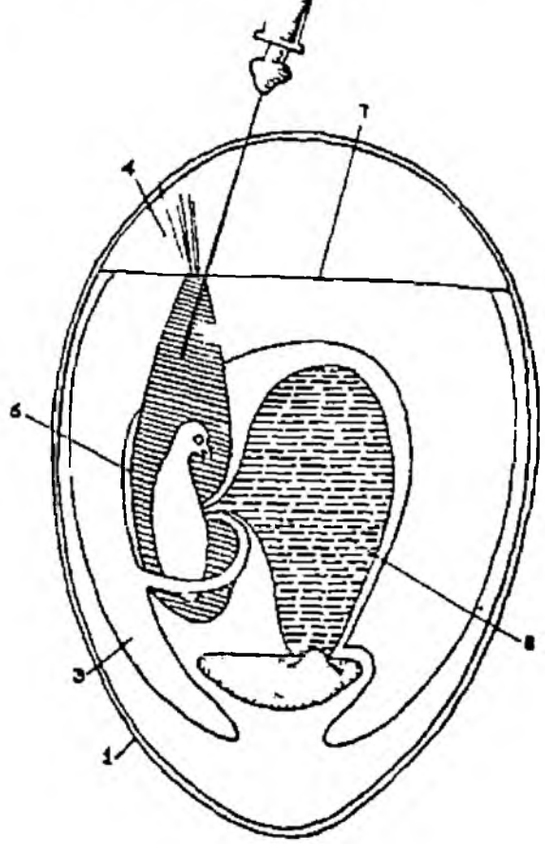


ALLANTOIC CAVITY INOCULATION



YOLK SAC INOCULATION

1 SHELL
2 CAM
3 ALLANTOIC CAVITY
4 AIR SPACE
5 ARTIFICIAL AIR SPACE
6 AMNIOTIC CAVITY
7 SHELL MEMBRANE
8 YOLK SAC



AMNIOTIC CAVITY INOCULATION

3.15. Field evaluation of the virus

3.15.1. Preliminary field experiment

A preliminary field trial using four concentrations of the PIBs and endosulfan (0.05 per cent) as a standard was conducted at the College of Agriculture, Vellayani, during 1983. Teepol 0.1 per cent was used for suspending the PIBs in spray fluid. The NPV used in the experiment was multiplied in *S. litura*. Each treatment was replicated four times.

Twenty four uniformly infested coconut palms (8 year old) were selected at random. Two hundred millilitres of the spray fluid was sprayed on each frond using a knapsac sprayer. The control palm received a spray with 200 ml of distilled water containing 0.1 per cent teepol only. Observations on larval mortality were recorded three days after treatment. Larvae found living at the time of observation were collected and reared in the laboratory for recording later mortality, if any. The pupal mortality and adult emergence were also recorded. The data were analysed statistically.

3.15.2. Main field experiment

To assess the efficacy of the virus under field condition a trial, using the concentration of PIBs which gave a mortality on par with that of endosulfan in the preliminary experiment viz.,22.14 x 10^7 PIBs/ml, was conducted along with endosulfan 0.05 per cent spray as standard. Control treated with teepol 0.1 per cent in water was also included. The experiment was conducted in some of heavily infested gardens in Quilon district. It was done in three different locations in the area.

The trees in each location were divided into three blocks of thirty each. Pre-treatment larval population was assessed by following the methods standardised by George et al., (1982). On each tree the fronds were numbered serially. Fronds between the lowest and upper most attacked ones, and the infested leaflets in them were counted. One fifth of the total infested leaflets were collected as sample at random. The larvae in the leaflets were recorded and the larval population per tree was assessed as follows:

$$\text{Larvae/tree} = \frac{\text{Pests in the leaflets sampled} \times \text{Total No. of attacked leaflets}}{\text{No. of leaflets taken as sample}}$$

The observations were repeated at weekly intervals for 14 weeks. The data were analysed statistically.

3.15.3. Estimation of natural parasitism

The extent of pupal parasitism in different plots of the above experiment was estimated by following the methods standardised by Pillai and Nair (1982). Thirty pupae collected at random from each plot at fifteen days interval were maintained in the laboratory individually in specimen tubes. The emergence of different parasitoids were observed and from the data the percentage of parasitism was worked out.

Thirty pupal cases of O. arenosella from which the moths/parasitoids had emerged were collected at random from each plot in the experimental area at fifteen days intervals. The pupal cases were brought to the laboratory and examined. Based on the size and position of emergence hole the pupae infected by Brachymeria sp. and Trichospilus sp. were identified, counted and calculated the percentages of incidence by the parasitoids.

RESULTS

RESULTS

4.1. Symptomatology

The symptoms of nuclear polyhedrosis infection in the larvae of Opisina arenosella Wlk. became evident in three to four days after ingestion of the virus. The infected larvae became paler than the healthy ones (Fig.5) and soon they became lethargic showed a loss of appetite. They were less responsive to tactile stimuli. A dark brown fluid was sometimes seen discharged through the mouth which moistened the larval galleries on the leaves. They moulted, though the duration between moults got prolonged.

Two to three days prior to death the infected larvae stopped feeding completely. In later stages of infection the body became very flaccid and they stopped their movement also. The cuticle became very fragile and the whole body content became liquified and milky white in colour which could be seen through the cuticle (Fig.6). The cuticle ruptured on the slightest pressure liberating the liquified body content containing the polyhedra in large numbers.

The infected larvae were found lying dead inside the leaf galleries and sometimes they were seen coming

Fig. 5 Healthy (top) and Diseased (bottom) larvae of Opisina arenosella (three days after inoculation with NPV)

Fig. 6 Larvae of Opisina arenosella just prior to death after inoculation of NPV.

FIG. 5.

FIG. 6

out of the galleries and lying on the leaf surface prior to death. The cadavers were found sticking to the leaf blade with the oozing body fluid. The body then developed a dark colour (Fig.7) and in 24 to 48 hours the whole body got deeply darkened (Fig.8).

Many of the larvae which ingested the polyhedra in the later stages moulted as prepupae or pupae. The diseased pupae were dark brown while the normal ones were light brown in colour. Some of the diseased pupae exhibited the typical symptom of larval pupal mosaic also. The body tissues of the infected pupae became disorganised, disintegrated and got liquffied in due course as in the case of the larvae. The body wall became very fragile and ruptured easily on touch liberating the body content with large number of polyhedral bodies. Larvae when fed with nuclear polyhedrosis virus in late fifth instar stage pupated in course of time and gave rise to malformed adults with short and ruffled wings (Fig.9). In some the moths were unable to come out of the pupal skin and died within the pupal case. Malformed adults lacked the normal colouration and their tissues and body fluids contained large number of polyhedral inclusion bodies.

Fig. 7 Larvae of Opisina arenosella immediately after death caused by NPV

Fig. 8 Larvae of Opisina arenosella 24 hour after death caused by NPV

FIG 7

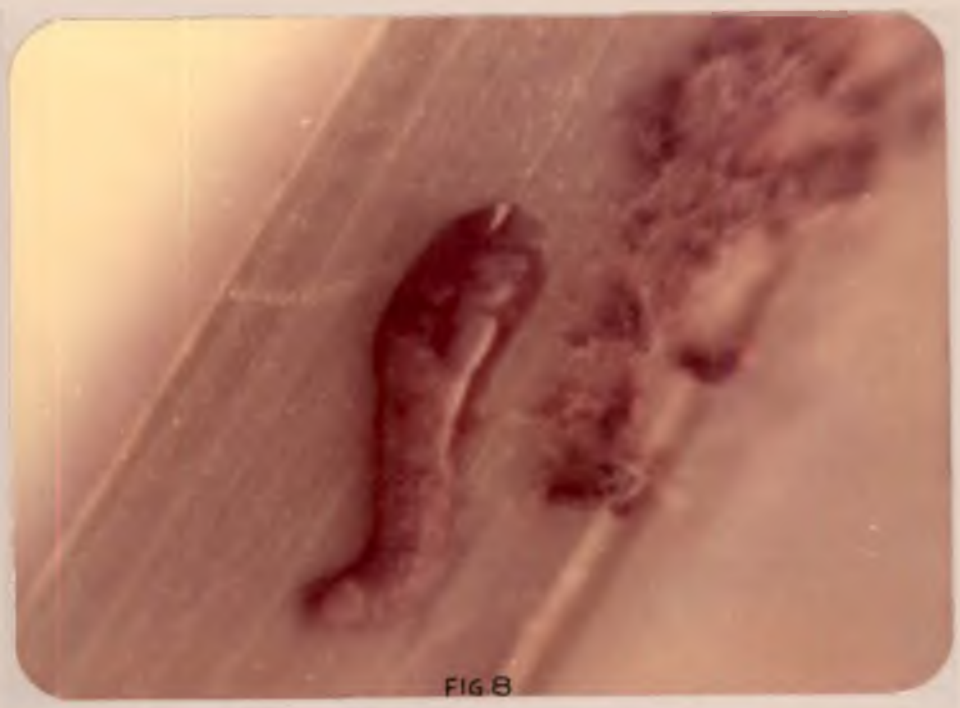


FIG 8

Fig. 9 Healthy moths of Oiketicus arenosella (left) and moths infected by NPV (right)

FIG. 9

4.2. Size and shape of polyhedra

The electron micrographs of the nuclear polyhedrosis virus of O. arenosella showed that the polyhedra were irregular in shape, varying considerably in size (Fig.10). The surface of the polyhedra was smooth without any ornamentation. The carbon replicas of the polyhedra did not show any surface pattern (Fig.11). The diameter ranged from 533.33 nm to 1666.67 nm with an average of 1393.30 nm (Fig.12). Section of the polyhedra showed that varying number of virus rods were enclosed in one developmental membrane and hence it was identified as a multiple embedded virus (Fig.13).

4.3. Histopathology

These histopathological studies were made on larvae killed at the end of 24, 48, 72, 96, 120 and 144 hours after inoculation with the virus. The course of infection as observed in the different tissues, with the aid of light microscope is presented below.

Twentyfour hours after inoculation

The polyhedral bodies were not visible in any of the tissues of infected larvae at this stage.

Fig. 10 Electron micrographs of polyhedra isolated from *Opisina arenosella* x 13000

FIG. 10

Fig. 11 Electron micrographs of carbon replica of polyhedra isolated from Oiaina arenosella x 54000

FIG.11

Fig. 12 Electron micrographs or sections of polyhedra isolated from Opsiina arenosella x 15000

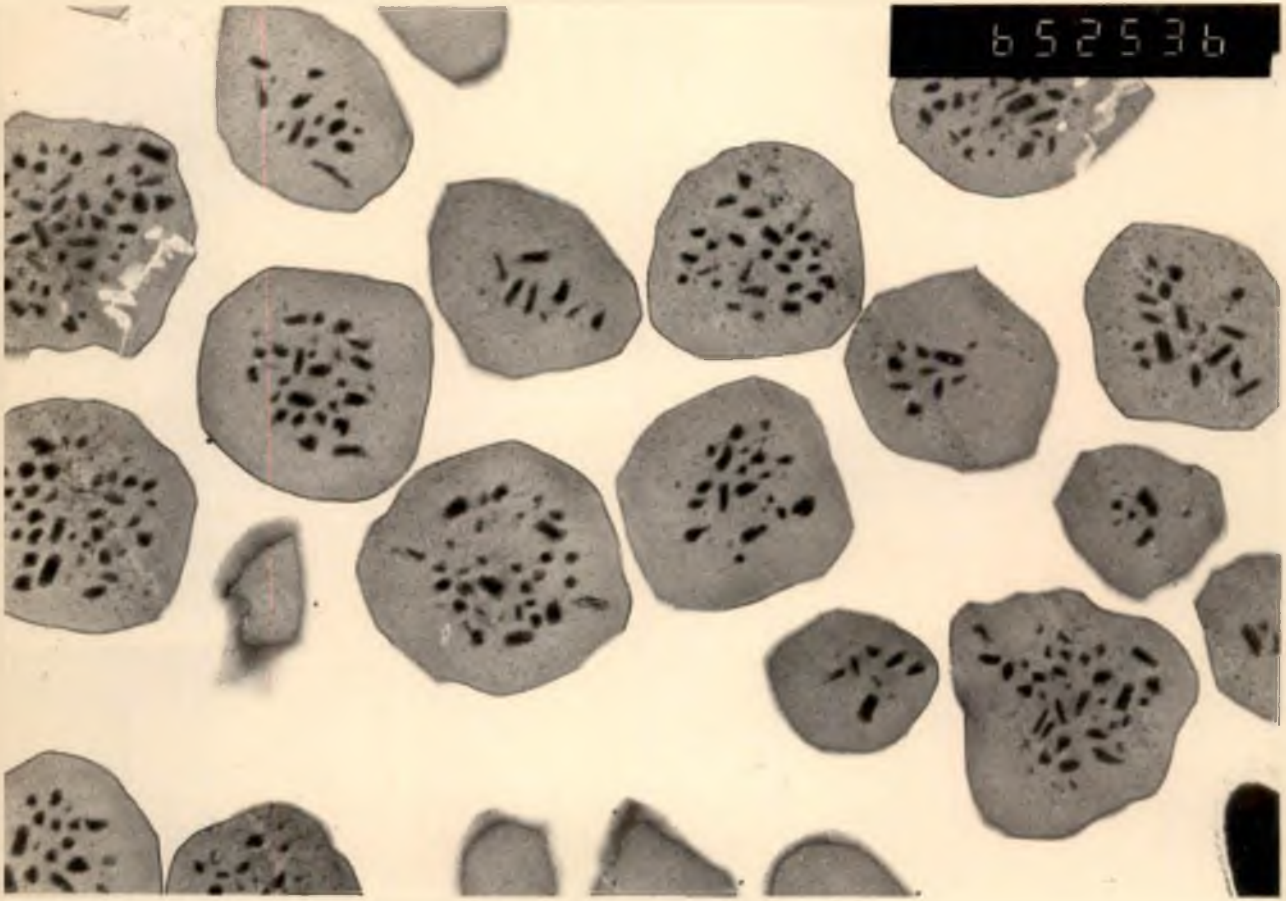


FIG.12

Fig. 13 Electron micrographs of section of polyhedra isolated from *Opisina arenosella* x 50000

D Developmental membrane

V Virions

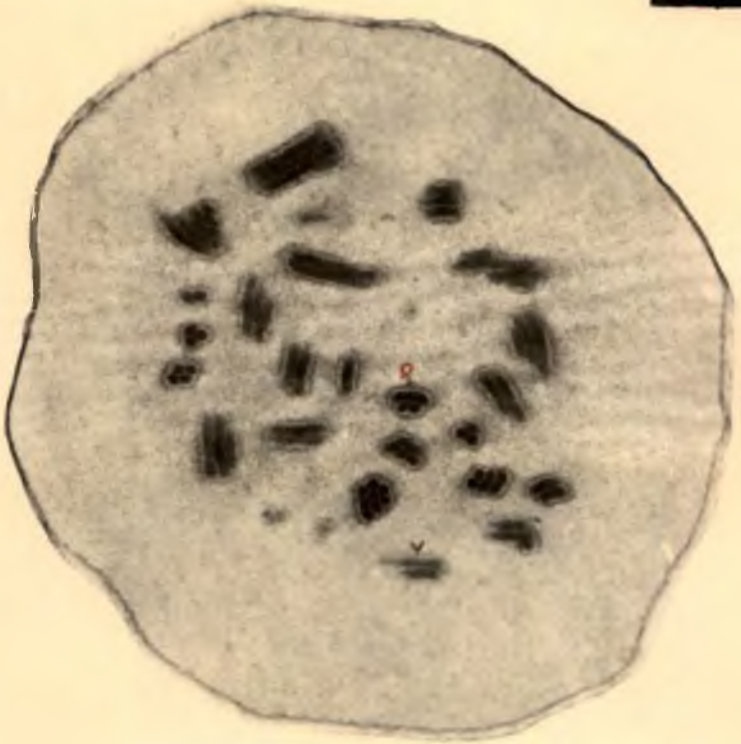


FIG. 13.

Fortyeight hours after inoculation

Many of the nuclei of hypodermis, fat bodies and some nuclei of trachea, connective tissues surrounding the gut and the gut epithelium were seen hypertrophied with clear ring zones at the periphery (Fig.14).

Seventytwo hours after inoculation

The infection progressed further and almost all the nuclei of hypodermis, fat body, trachea, connective tissues surrounding the gut and gut epithelium were seen conspicuously hypertrophied (Fig.15). The Malpighian tubules, nerve ganglia and muscles were also seen infected, but it was only in the initial stage.

Ninetysix hours after inoculation

In the cells of hypodermis, fat body, trachea, connective tissues surrounding the gut and in gut epithelium the infection had progressed further and fully developed polyhedra were visible in the nuclei(Fig.16). The infection in Malpighian tubules, nerve gangalia and muscles also progressed further. Many cells of the epithelial sheath of the gonads showed early signs of infection.

Fig. 14 Longitudinal sections of O.arenosella larva fixed at 48 hours after inoculation with the NPV x 50

H - Hypodermis
F - Fat body
T - Trachea
G - Gut epithelium

Fig. 15 Longitudinal sections of O. arenosella larva fixed at 72 hours after inoculation with the NPV x 50

H - Hypodermis
F - Fat body
T - Trachea
G - Gut epithelium
M - Muscles
CT - Connective tissues
MT - Malpighian tubules

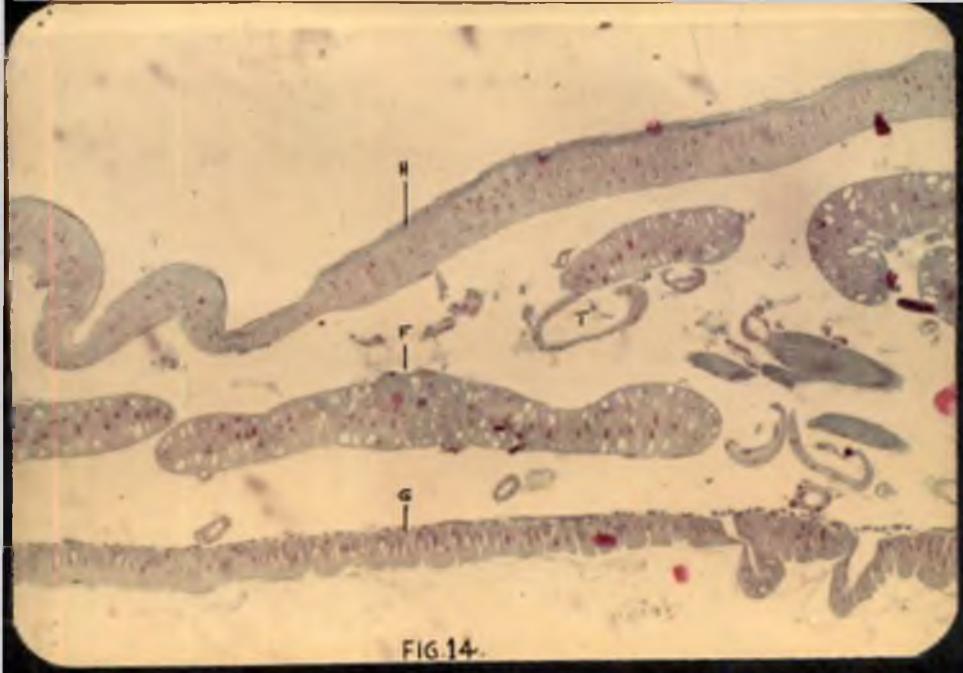


FIG. 14.

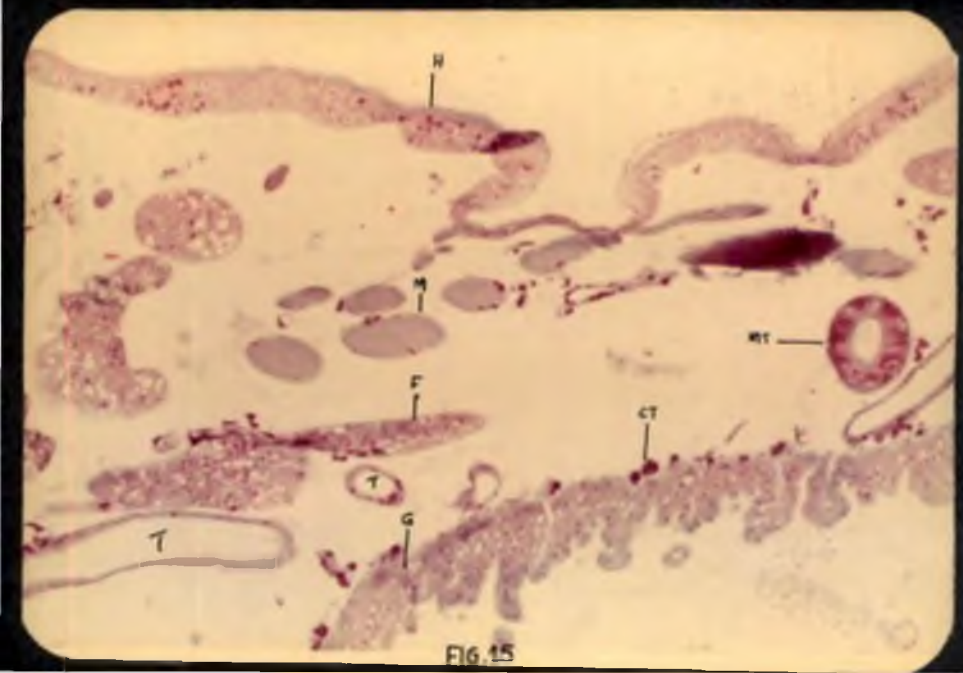


FIG. 15

One hundred and twenty hours after inoculation

The infection had spread completely over hypodermis, fat body, trachea and epithelial sheath of gonads. Fully developed polyhedra were visible in many cells (Fig.17). The infection had advanced in Malpighian tubules, nerve ganglia and muscles too.

One hundred and fortyfour hours after inoculation

At this stage many tissues (hypodermis, fat body, trachea) showed signs of disintegration. Fat bodies were seen heavily loaded with polyhedra and some of them even ruptured releasing the polyhedra into the haemocoel (Fig.18). Cells of muscles, nerve ganglia, Malpighian tubules, epithelial sheath of gonads and connective tissues surrounding the gut were also seen in the advanced stage of infection.

The precise histopathological changes observed in the different tissues are detailed below.

Hypodermis

Photomicrographs of infected hypodermis at different intervals after inoculation are presented in Figs.19 to 23. The normal hypodermis consisted of a single layer of cells,

Fig. 16 Longitudinal sections of O. arenosella larva at 96 hours after inoculation with NPV X50

H - Hypodermis
F - Fat body
T - Trachea
G - Gut epithelium
M - Muscles
CT - Connective tissues

Fig. 17 Longitudinal sections of O. arenosella larva at 120 hours after inoculation with NPV X50

H - Hypodermis
F - Fat body
T - Trachea
GO - Gonads

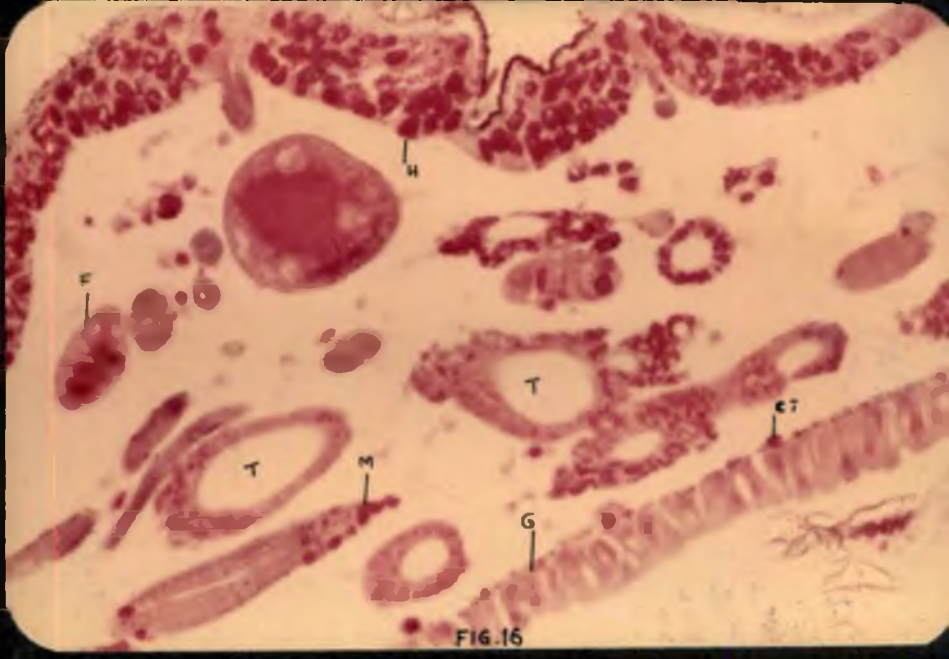


FIG. 16

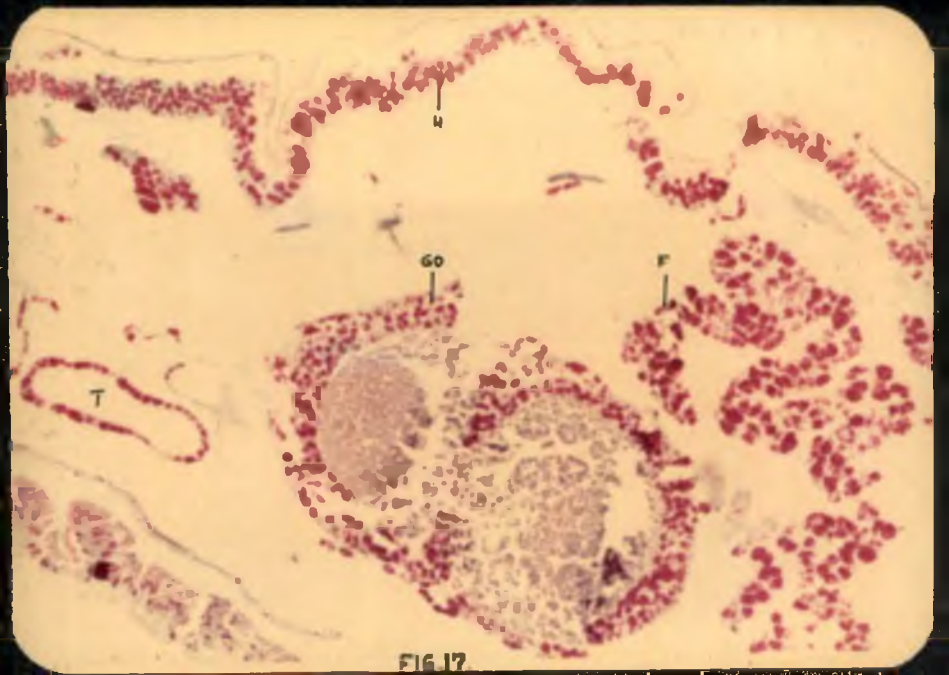


FIG. 17

Fig. 18 Longitudinal section of O. arenosella larva at 144 hours after inoculation with NPV X50

H - Hypodermis

F - Fat body

T - Trachea

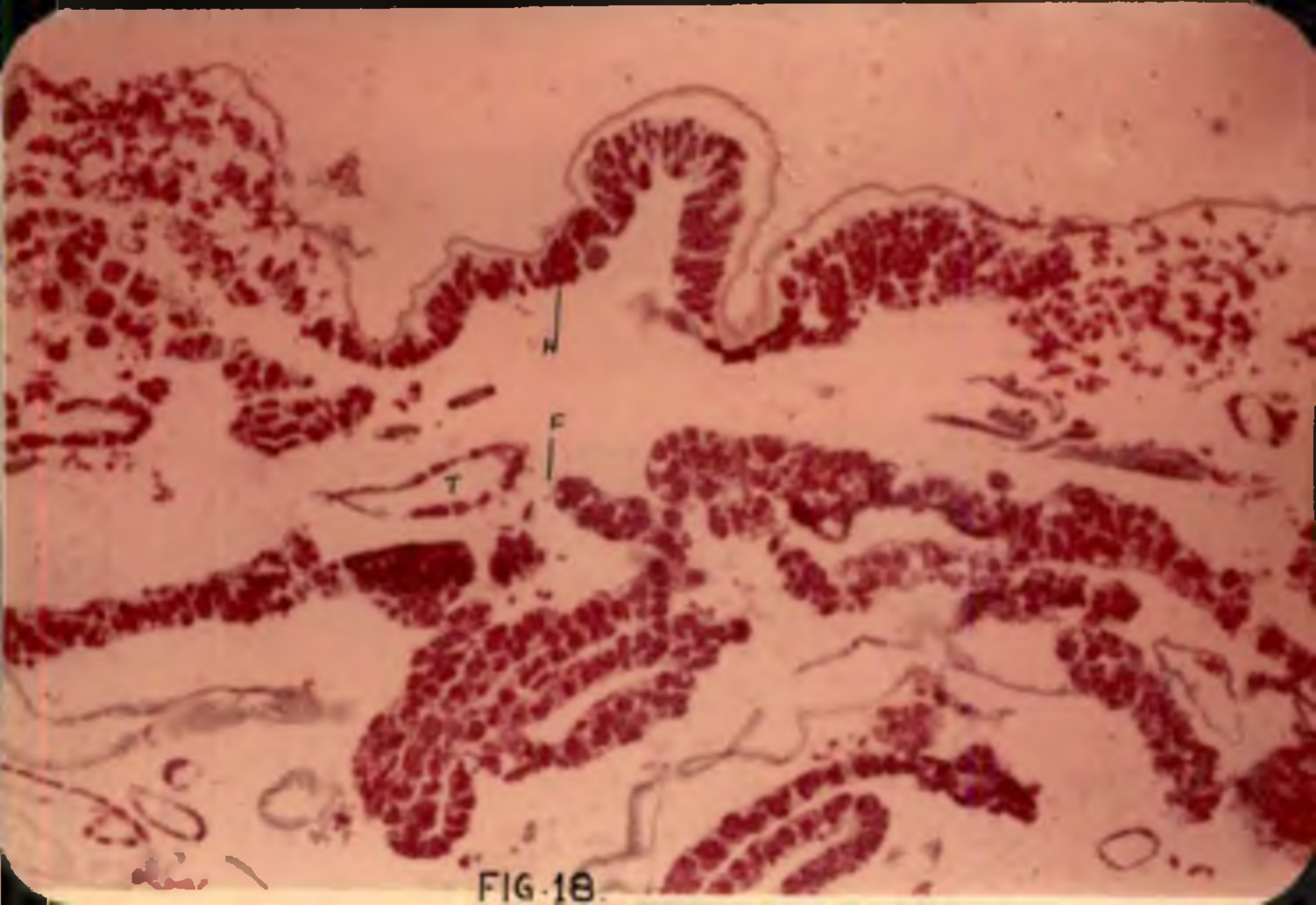


FIG. 18.

resting on a basement membrane. No apparent changes were noticeable with light microscope at 24 hours after inoculation. Early signs of infection were observed in the hypodermis of larvae fixed at 48 hours after inoculation. At this stage (Fig.19) a limited number of the nuclei in the hypodermis were seen hypertrophied and they showed a chromatin condensation resulting in the formation of unified masses at the centre called 'Virogenic stroma' (Xeros, 1956) with clear 'ring zones' at the peripheral area. The hypodermal layer itself appeared swollen in most regions. The infected tissues measured 2450 nm in thickness (mean) with a range of 1980 nm to 3020 nm.

In larvae fixed at 72 hours after ingestion of the polyhedra most of the hypodermal cells had hypertrophied nuclei and the hypodermis appeared considerably swollen (Fig.20). On an average the infected tissues measured 3560 nm in thickness with a range of 2970 nm to 4120 nm.

At 96 hours after inoculation the hypertrophied nuclei enlarged further and completely filled the cells and the location of the nuclei with polyhedra in different levels gave it a stratified appearance (Fig.21).

In larvae fixed at 120 hours after ingestion of the polyhedra, the cells were elongated and in many regions

Fig. 19 Section of the hypodermis of O. arenosella fixed at 48 hours after inoculation with NPV X200

H - Hypodermis

HN - Hypertrophied nuclei

R - Ring zone

Fig. 20 Section of the hypodermis of O. arenosella fixed at 72 hours after inoculation with NPV X200

H - Hypodermis

R - Ring zone

HN - Hypertrophied nuclei

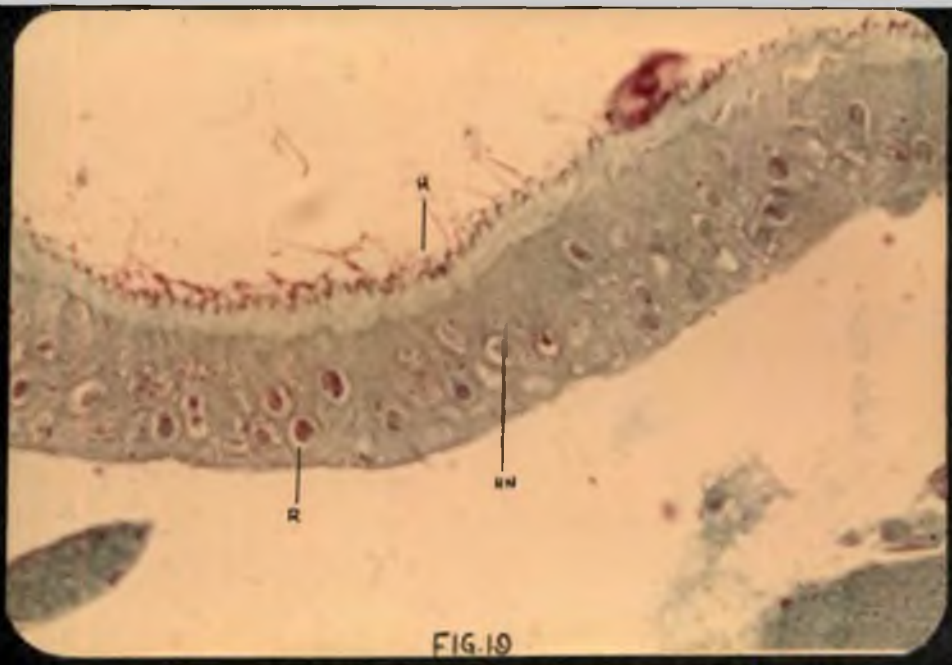


FIG.19

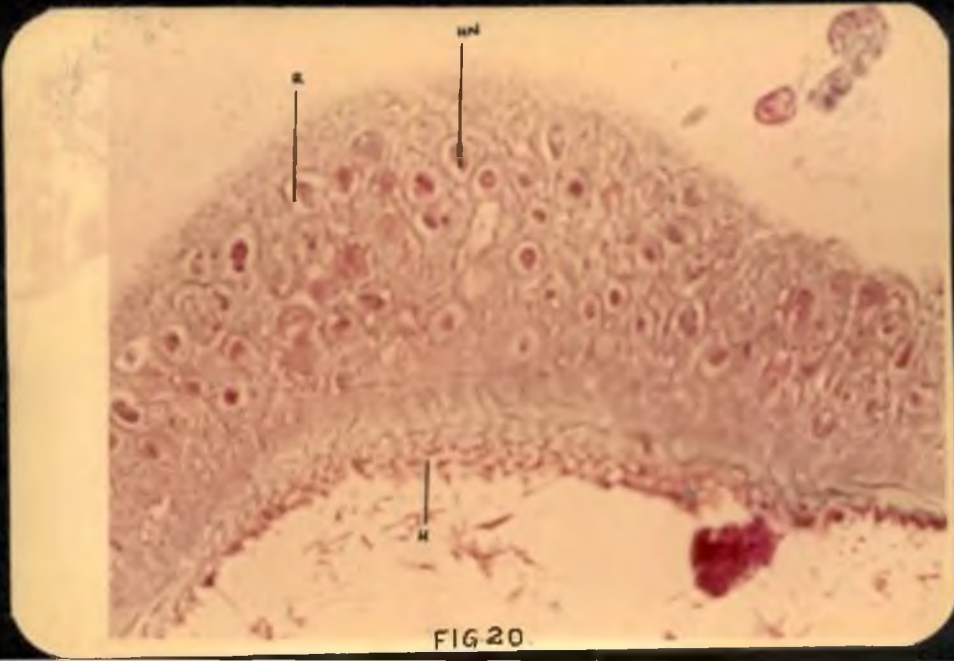


FIG 20

the signs of disintegration were visible (Fig.22). Fully developed polyhedra were present in large numbers in the nuclei.

At 144 hours after inoculation the hypodermis had more or less completely retracted from the cuticle in many regions. The cells showed clear signs of disintegration and the cell boundaries were lost (Fig.23). The cells were seen ruptured liberating the polyhedra into the haemocoel.

Fat body

The changes observed in the adipose tissues during the course of nuclear polyhedrosis virus infection are illustrated in Figs. 24 to 28.

The normal fat body cells are characterised by the presence of large number of fat vacuoles in their cytoplasm. By 48 hours after inoculation the nuclei of a limited number of cells were seen hypertrophied and in some of these the chromatin had condensed to form central 'Virogenic stroma' with 'ring zone' around (Fig.24). Fat vacuoles were fewer in number and smaller in size.

Fig.21 Section of the hypodermis of O. arenosella fixed at 96 hours after inoculation with NPV X200

H - Hypodermis

N - Nucleus

Fig. 22 Section of the hypodermis of O. arenosella fixed at 120 hours after inoculation with NPV X200

C - Cuticle

H - Hypodermis

N - Nucleus

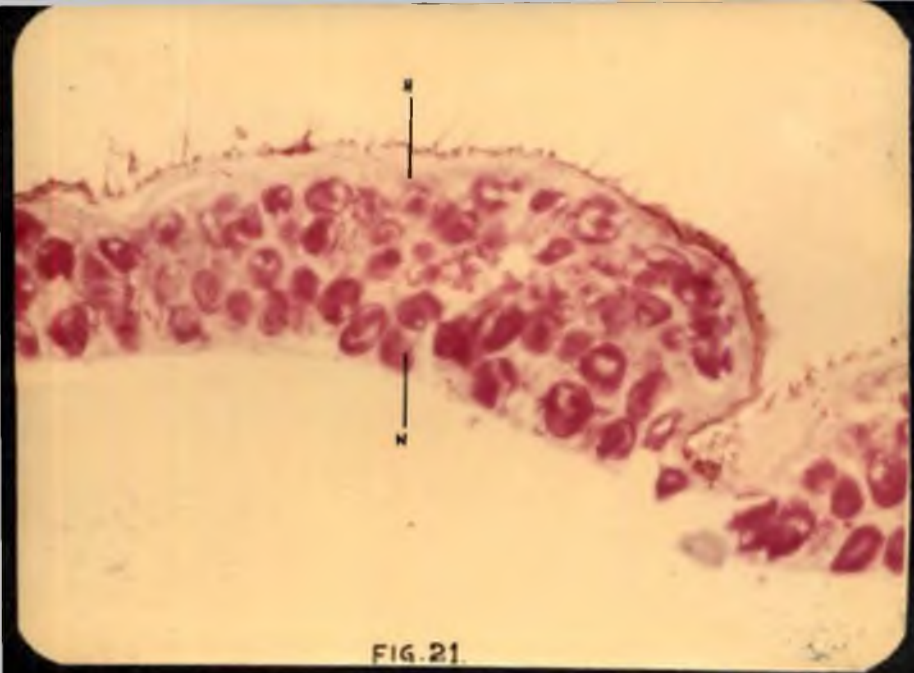


FIG. 21.

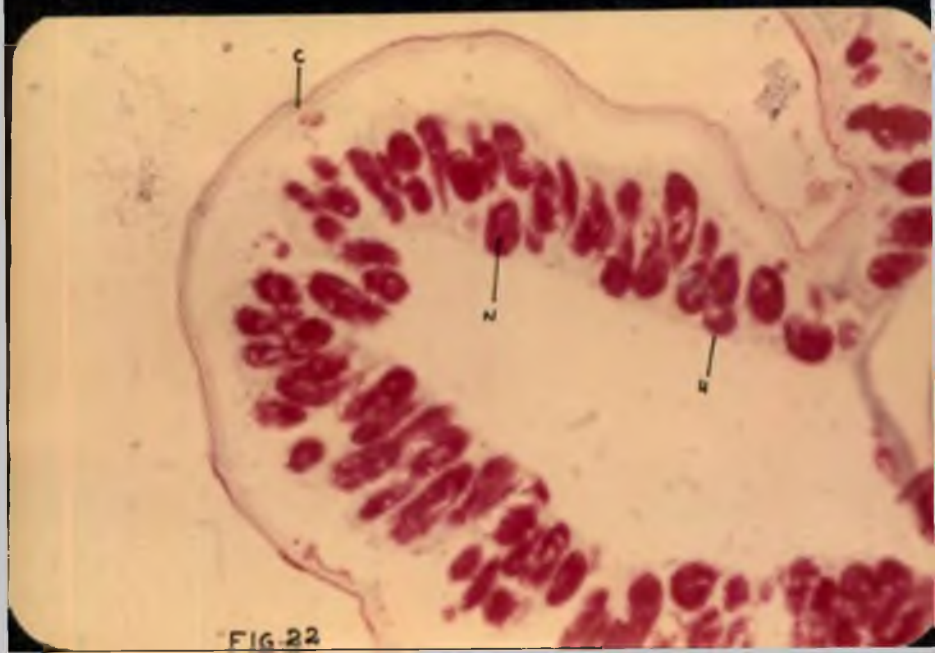


FIG. 22

Fig. 23 Section of the hypodermis of O. arenosella fixed at 144 hours after inoculation with NPV X200

P - Polyhedra
C - Cuticle
N - Nucleus
H - Hypodermis

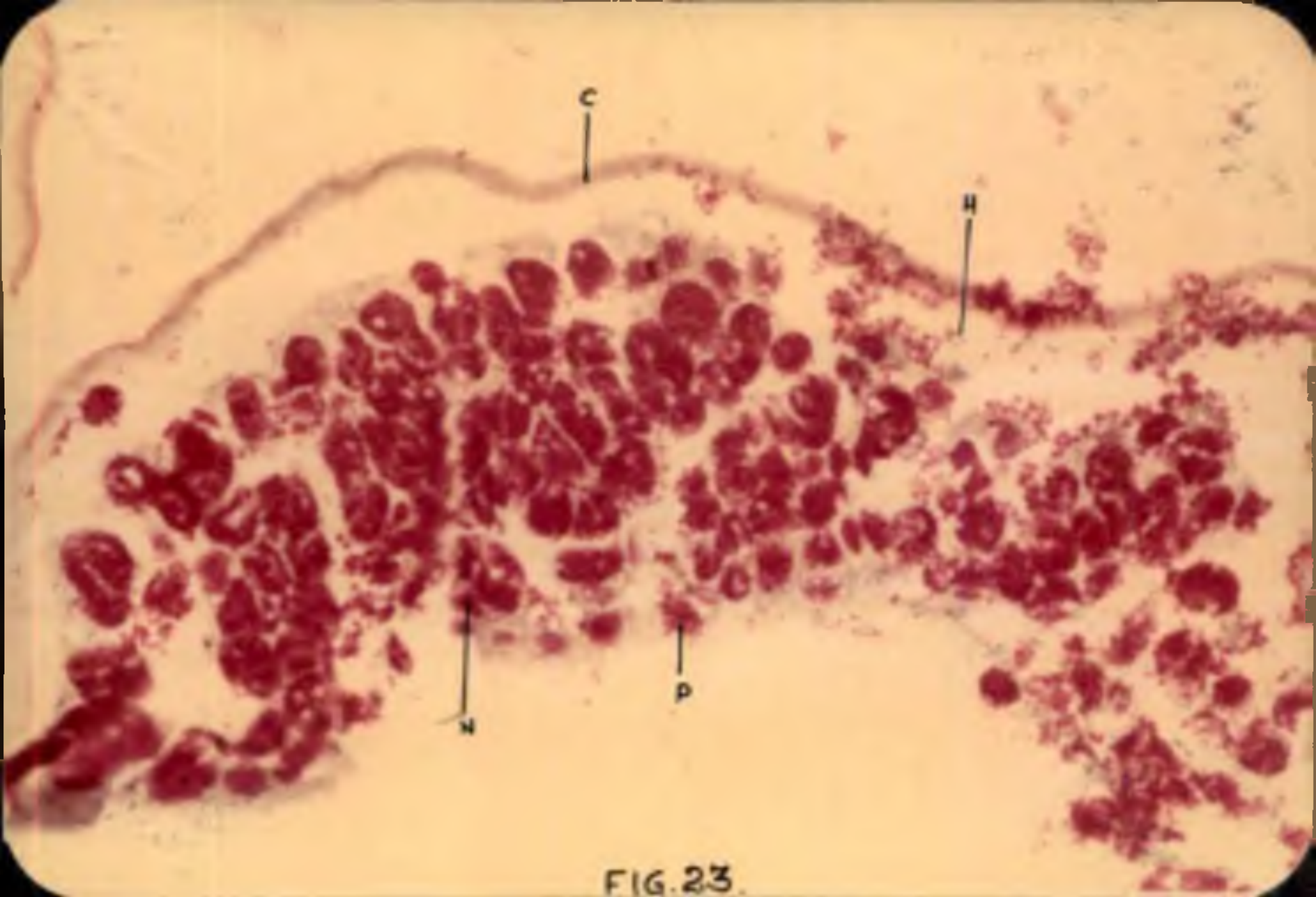


FIG. 23.

In larvae fixed at 72 hours after inoculation the hypertrophied nuclei of infected cells enlarged further and completely filled the cell membrane. The nuclei at various stages of infection could be seen in different lobes of adipose tissues. Even the adjacent cells could be seen in widely different stages of inection (Fig.25). The number of hypertrophied nuclei had increased considerably. The fat vacuoles were very few in number and small in size.

At 96 hours after inoculation, the nuclei appeared as dark staining masses almost completely filling the cells (Fig.26). Many nuclei had fully developed polyhedra inside. The hypertrophied nuclei increased considerably in number and fat vacuoles were very few in the infected areas.

In larvae fixed at 120 hours after ingestion of the virus, the infection spread to move nuclei and fully formed polyhedra were present in majority of them (Fig.27). Even at this stage nuclei at different stages of infection could be seen and the fat vacuoles showed a very marked reduction in number.

Fig. 24 Section of the adipose tissue of O. arenosella fixed at 48 hours after inoculation with NPV X200

F - Fat body
HN - Hypertrophied nuclei
M - Muscle
R - Ring zone
V - Vacuoles

Fig. 25 Section of the adipose tissue of O.arenosella fixed at 72 hours after inoculation with NPV X200

F - Fat body
N - Nucleus
V - Vacuoles
R - Ring zone

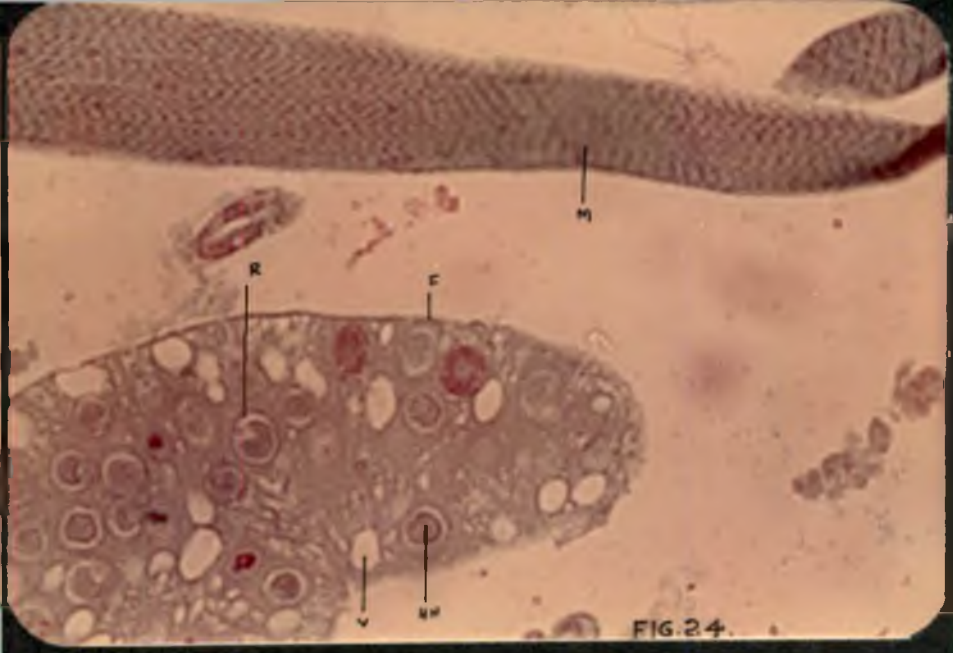


FIG. 24.

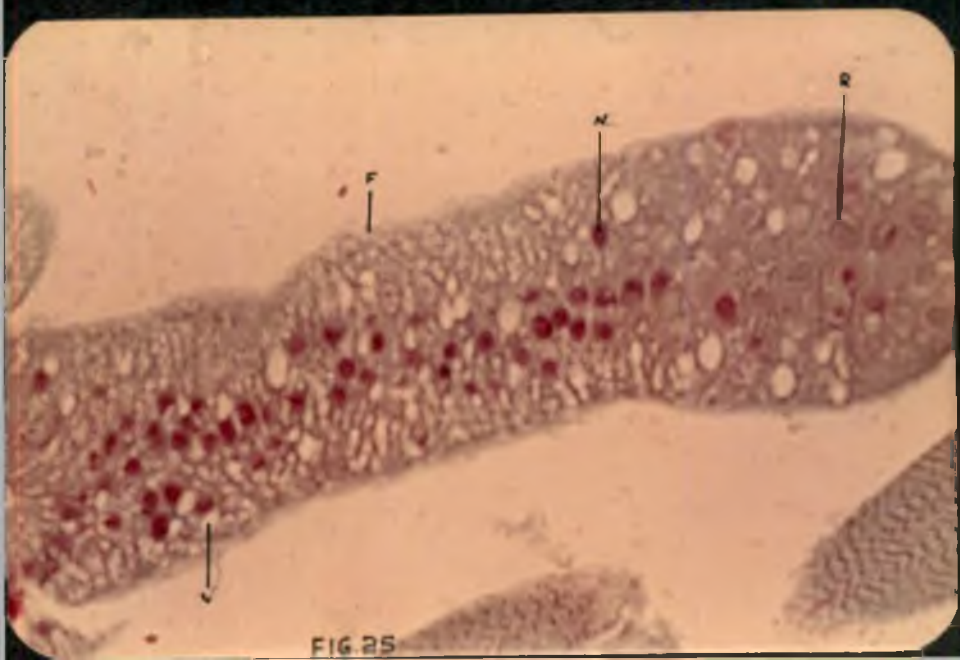


FIG. 25

Fig. 26 Section of the adipose tissues of O. aronosella fixed at 96 hours after inoculation with NPV X200

F - Fat body
N - Nucleus
P - Polyhedra
V - Vacuoles

Fig. 27 Section of the adipose tissues of O. aronosella fixed at 120 hours after inoculation with NPV X200

F - Fat body
P - Polyhedra
N - Nucleus
V - Vacuoles

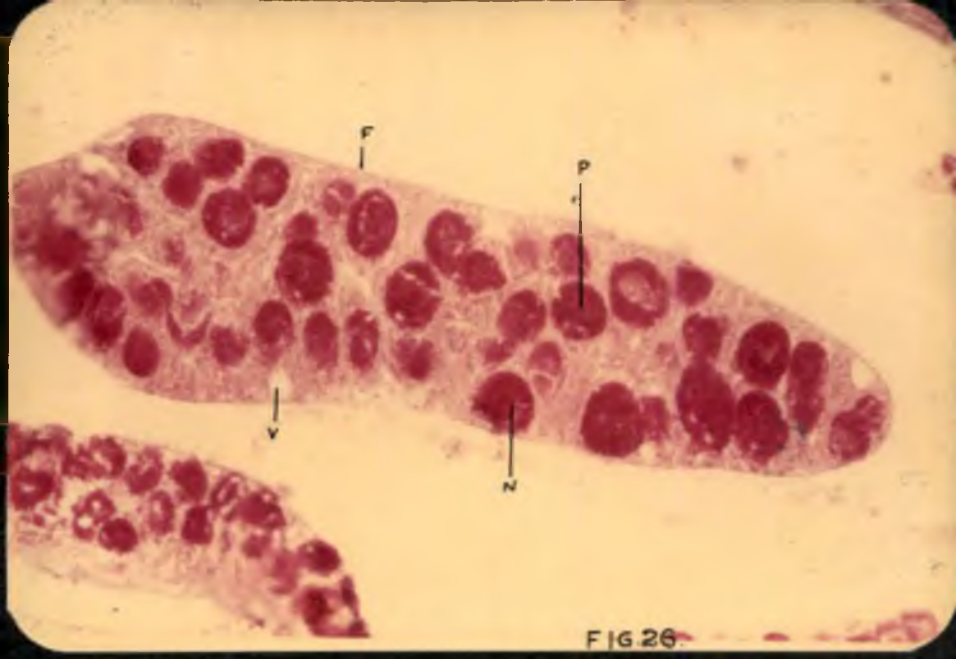


FIG 26.

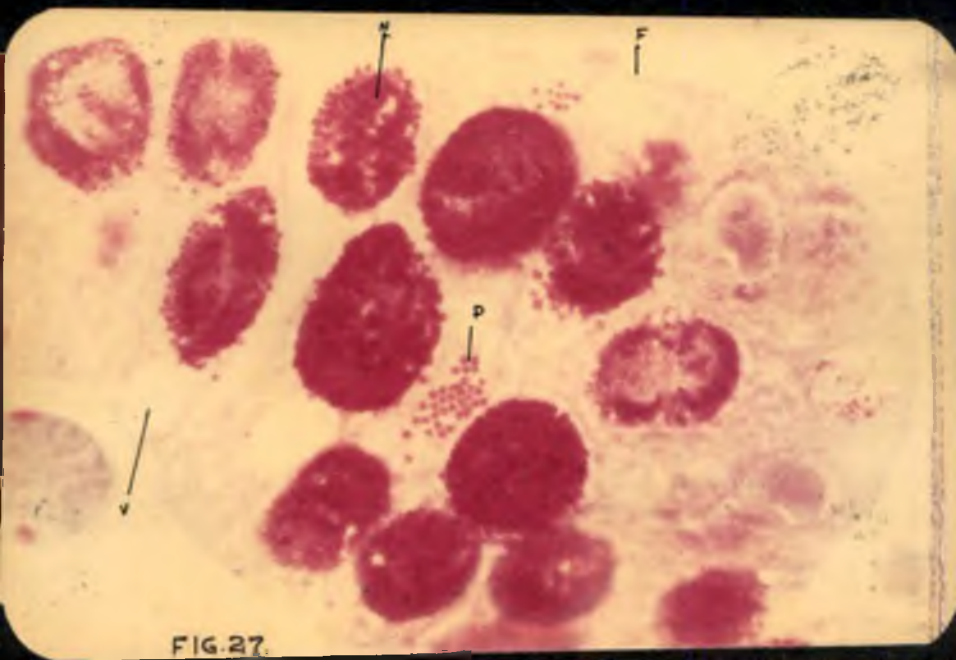


FIG 27.

At 144 hours after inoculation almost all the fat body cells were seen fully packed with polyhedra and fat vacuoles had completely disappeared. Disintegration of the tissue was conspicuous in many lobes and constituent cells had got separated from each other. Rupturing of the cells and liberation of polyhedra were also seen in many lobes of the fat bodies (Fig.28).

Muscle tissues

Infection of muscles or muscle sheath was not observed upto 48 hours after inoculation. At 72 hours after inoculation the polyhedral formation could be seen in the nuclei disposed immediately beneath the muscle sheath (Fig.29) as well as those placed deep in the sarcoplasm between the fibrillae. The infection had fairly advanced at 96 hours after inoculation and the polyhedra were distinctly visible below the sarcolemma (Fig.30).

In larvae fixed at 120 hours after inoculation, the infection had advanced further with dense group of polyhedra seen below the sarcolemma which appeared loosened from the underlying cells (Fig.31). The polyhedra increased in number and size and many cells of muscle showed signs

Fig. 28 Section of the adipose tissues of O. arenosella fixed at 144 hours after inoculation with NPV X200

P - Polyhedra
N - Nucleus
RL - Rupturing and liberation of polyhedra

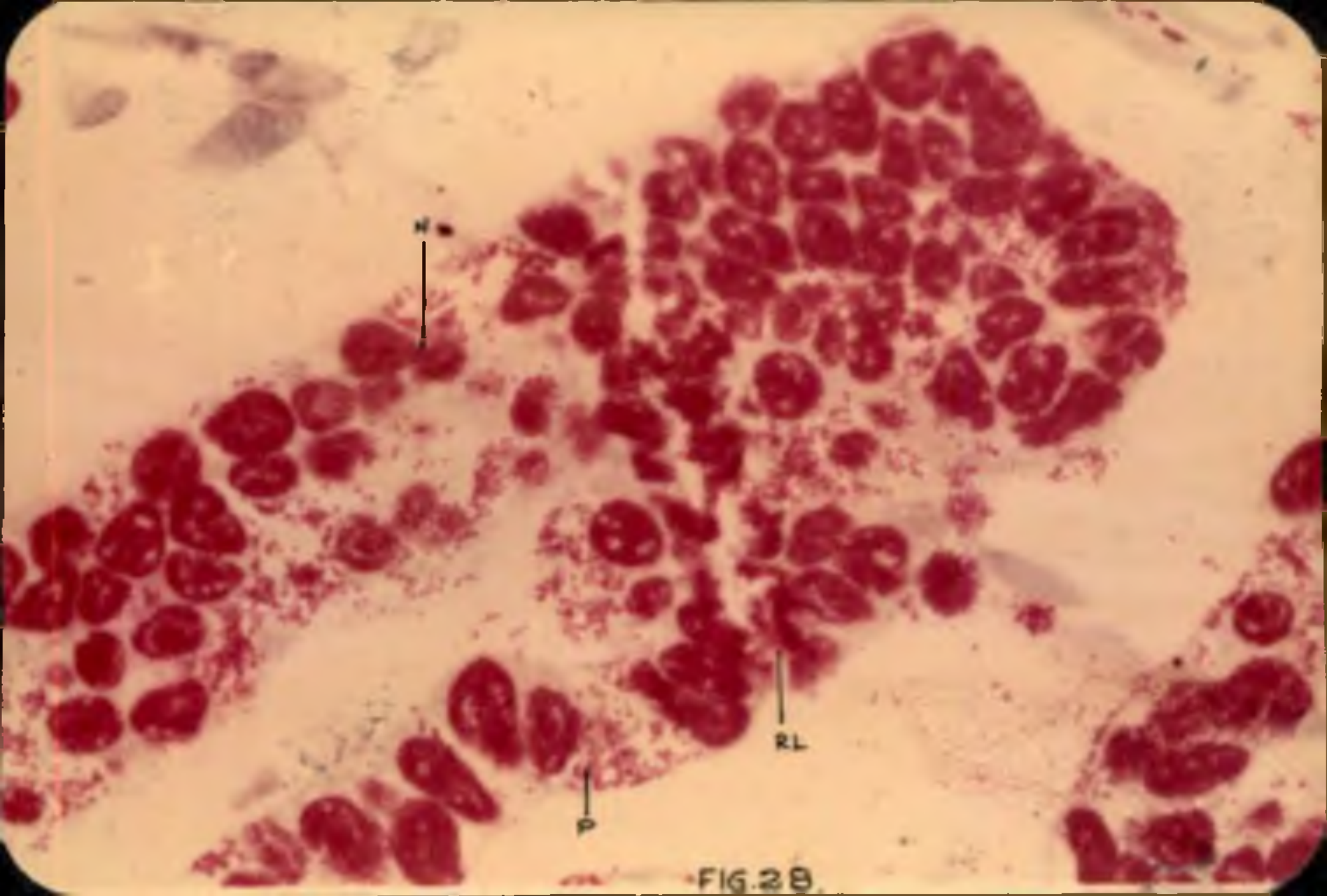


FIG. 28

Fig.29 Section of the muscle of O. arenosella fixed at 72 hours after inoculation with NPV X200

M - Muscle
I - Infection
MS - Muscle sheath

Fig.30 Section of the muscle of O. arenosella fixed at 96 hours after inoculation with NPV X200

M - Muscle
MS - Muscle sheath
P - Polyhedra.

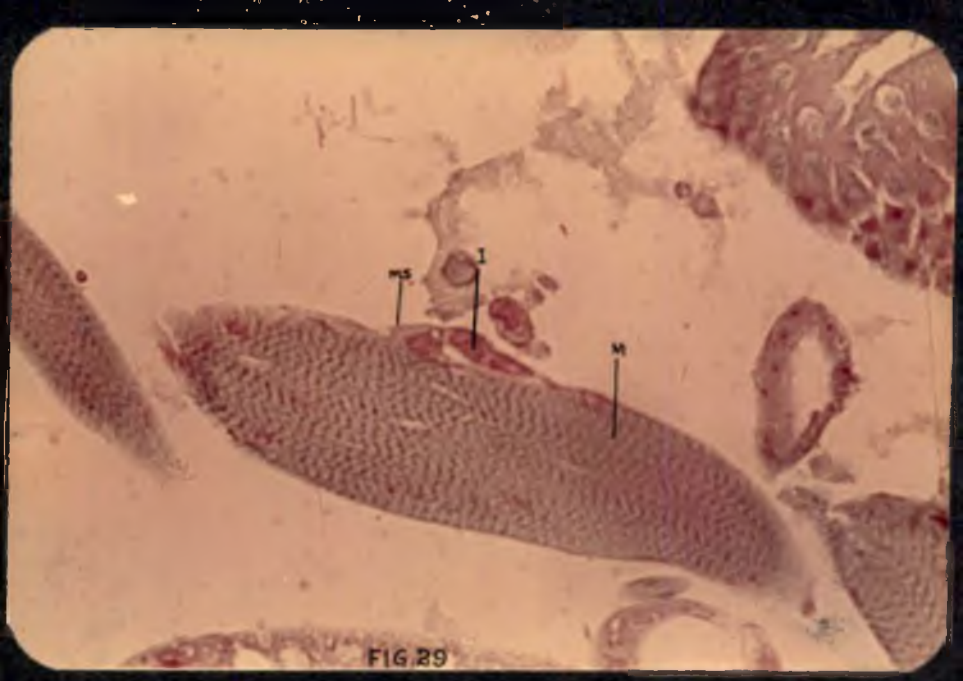


FIG. 29

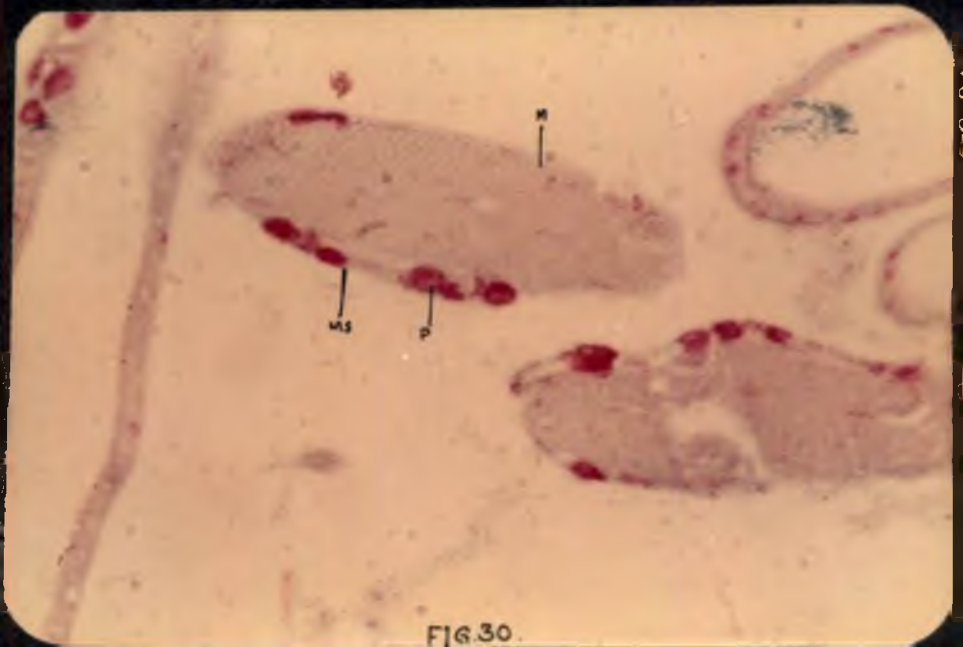


FIG. 30

of rupturing and liberation of polyhedra (Fig.32) at 144 hours after inoculation.

Tracheal matrix

At 24 hours following inoculation no indication of infection could be observed in the trachea. At 48 hours after inoculation nuclei of tracheal matrix cells throughout the body appeared conspicuously enlarged with clear 'ring zones' (Fig.33).

The infection had advanced and polyhedra were distinctly visible in most cells of trachea at 72 hours after inoculation (Fig. 34). The nuclei enlarged further as the polyhedra increased in number at 96 hours after inoculation (Fig.35).

At 120 hours after inoculation, fully developed polyhedra were seen in most of the nuclei and cells started rupturing liberating the polyhedra into the surrounding tissues (Fig.36). The cells disintegrated extensively at 144 hours after inoculation (Fig. 37). The polyhedra were liberated into the haemocoel in large numbers.

Fig. 31 Section of the muscle of O. arenosella fixed at 120 hours after inoculation with NPV X200

M - Muscle
P - Polyhedra

Fig.32 Section of the muscle of O. arenosella fixed at 144 hours after inoculation with NPV X500

P - Polyhedra
M - Muscle

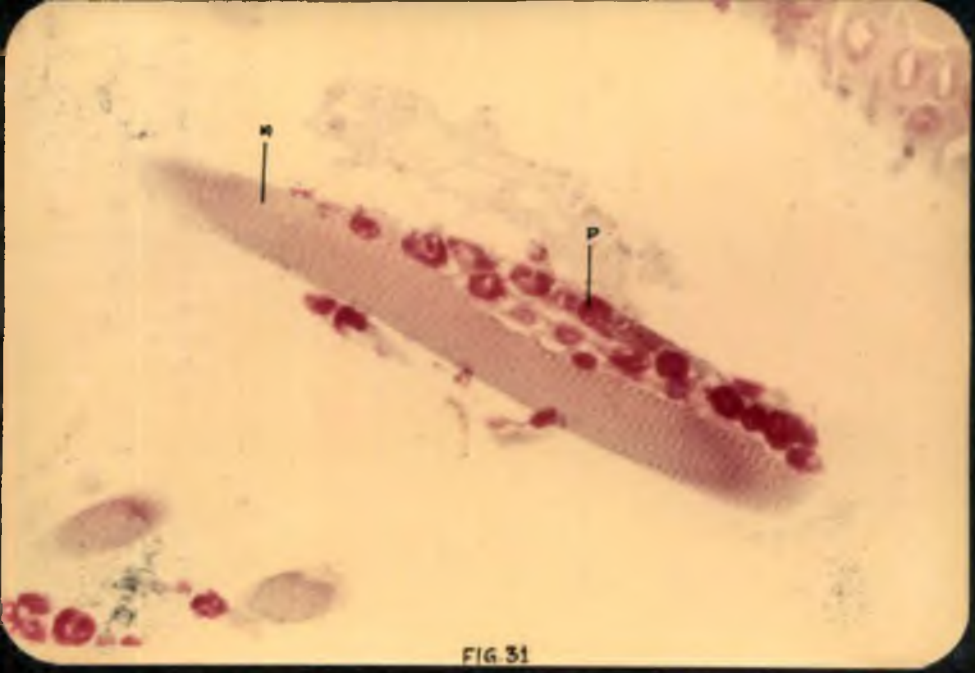


FIG 31

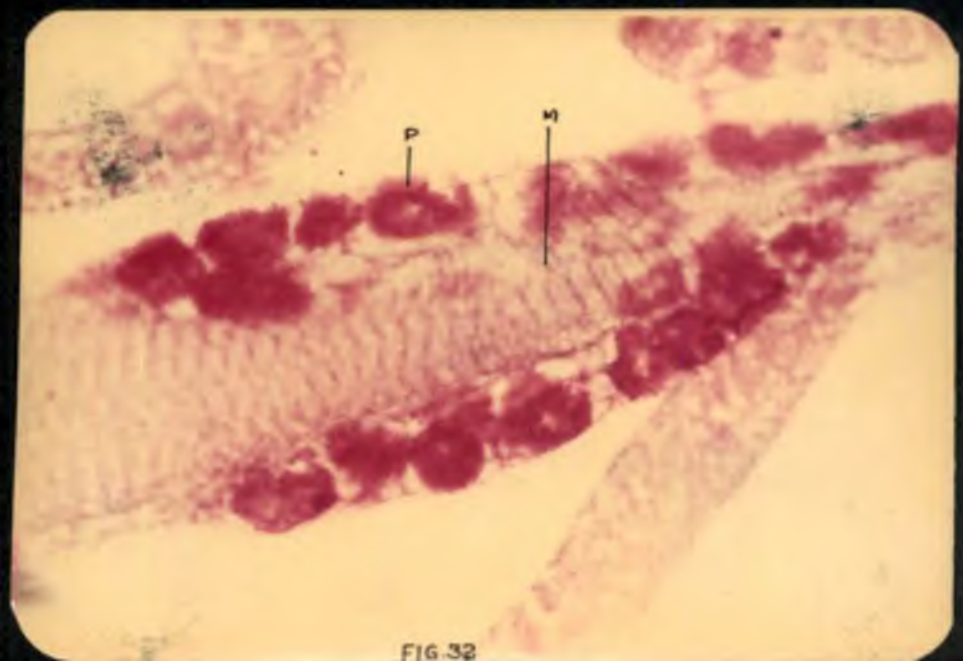


FIG 32

Fig. 33 Section of the trachea of *O. pteronotella* fixed at 48 hours after inoculation with NPV X500

T - Trachea
HM - Hypertrophied muscle
R - Ring zone

Fig. 34 Section of the trachea of *O. pteronotella* fixed at 72 hours after inoculation with NPV X500

P - Polyhedra
T - Trachea
N - Nucleus

FIG 33

FIG 34

Fig. 35 Section of the trachea of *O. arenosella* fixed at 96 hours after inoculation with NPV X200

T - Trachea
P - Polyhedra
N - Enlarged nucleus

Fig. 36 Section of the trachea of *O. arenosella* fixed at 120 hours after inoculation with NPV X200

T - Trachea
P - Polyhedra
R - Rupturing and liberation of polyhedra

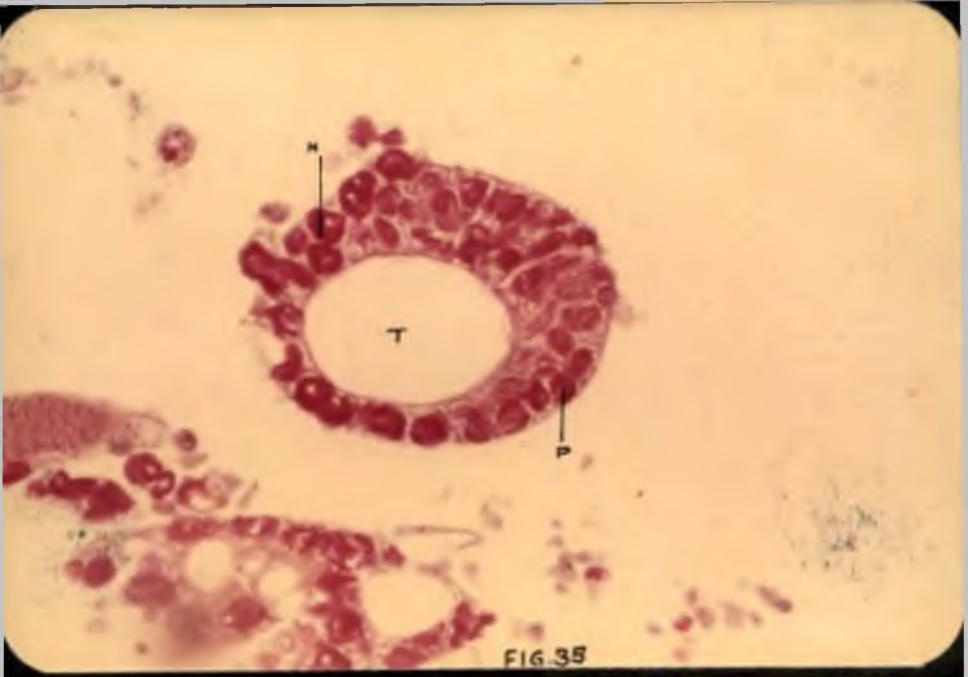


FIG. 35

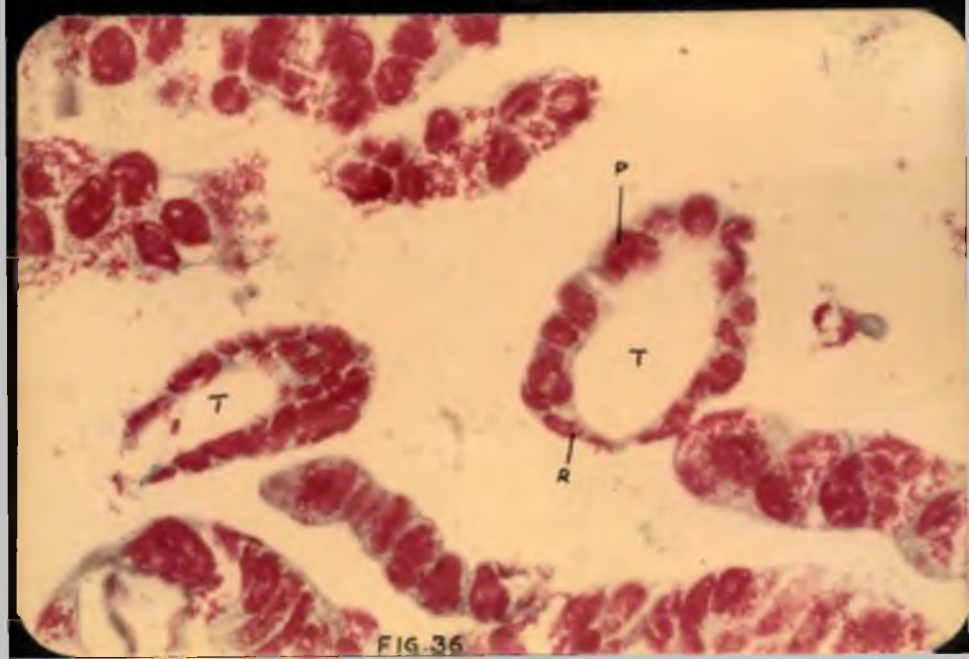


FIG. 36

Fig. 37 Section of the trachea of O. aranogella fixed at 144 hours after inoculation with NPV X200

T - Trachea
P - Polyhedra
RL - Rupturing and liberation of polyhedra

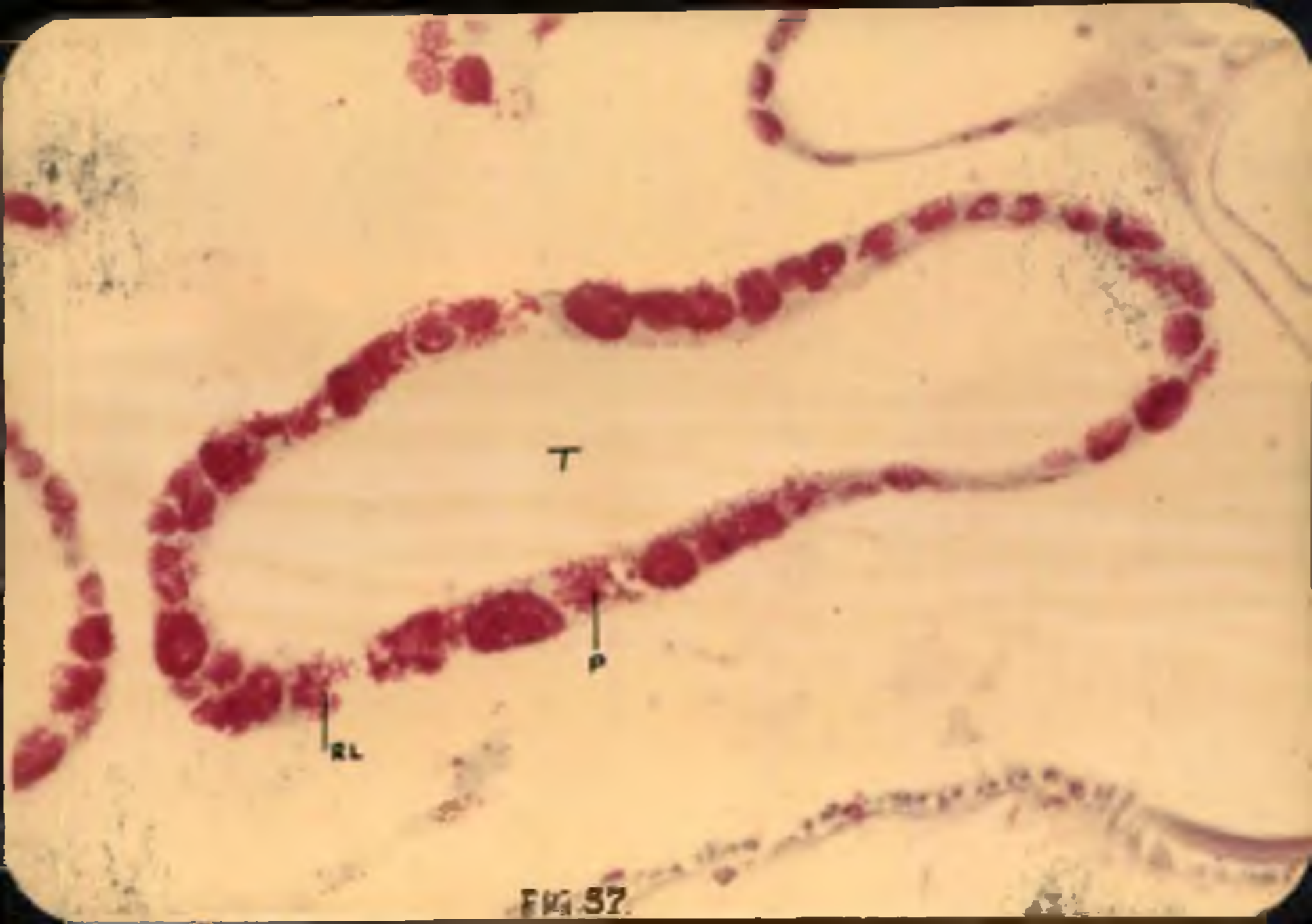


FIG. 57.

The gut epithelium and the connective tissues surrounding it

There was no apparent difference between the enteric epithelium and connective tissues of healthy and infected larvae for the first 24 hours after inoculation.

At 48 hours after inoculation some cells of gut epithelium showed signs of viral infection by their hypertrophied nuclei. Infection was also noticed in the connective tissues surrounding the gut(Fig. 38).

Polyhedra were distinctly visible in most cells of connective tissues surrounding the gut epithelium and in some cells of gut epithelium at 72 hours after inoculation (Fig.39). The infection appeared to be in the matrix cells of trachea associated with the connective tissues.

At 96 and 120 hours after inoculation many cells of connective tissues surrounding the gut epithelium showed advanced stage of infection (Figs.40 and 41). In many cells the polyhedra were distinctly visible at this stage.

At 144 hours after inoculation most of the cells showed signs of disintegration and rupturing and liberation of polyhedra into the haemocoel (Fig. 42).

Fig. 38 Section of the connective tissues surrounding the gut and gut epithelium of O. arenosella fixed at 48 hours after inoculation with NPV X200

CT --Connective tissues
G --Gut epithelium
N --Nucleus

Fig. 39 Section of the connective tissues surrounding the gut and gut epithelium of O. arenosella fixed at 72 hours after inoculation with NPV X200

P -- Polyhedra
CT -- Connective tissues
G -- Gut epithelium

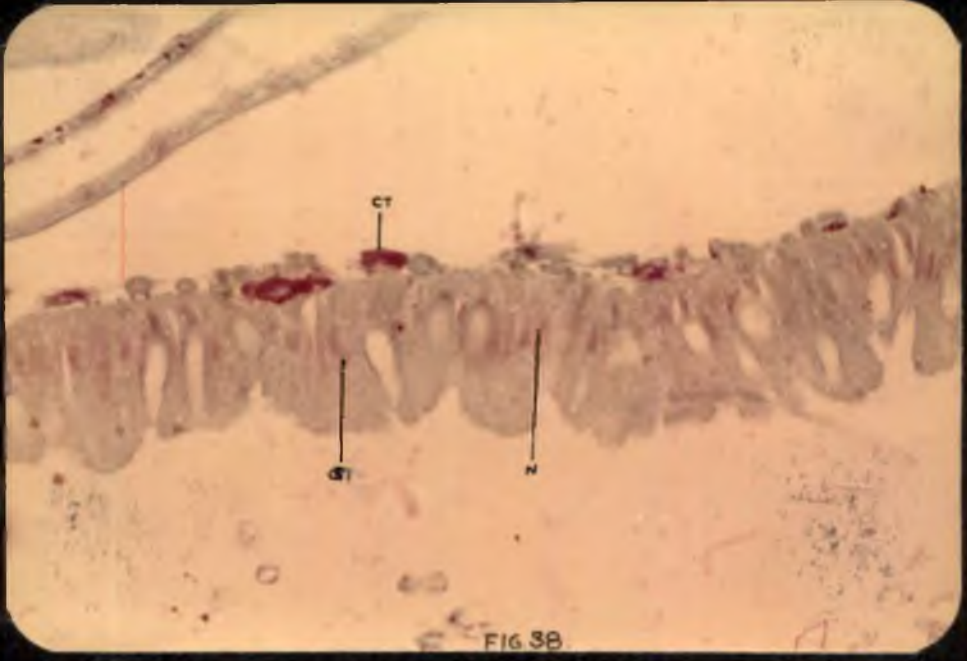


FIG 38

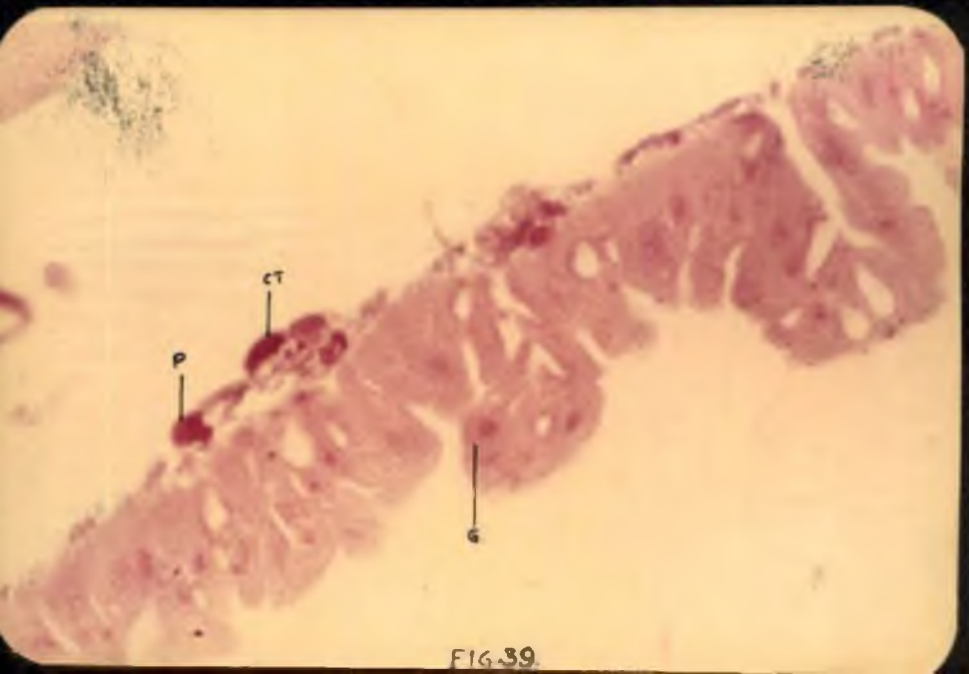


FIG 39

Fig. 40 Section of the connective tissues surrounding the gut and gut epithelium of O. <u>grenosella</u> fixed at 96 hours after inoculation with NPV X200

CT - Connective tissues
G - Gut epithelium
N - Nucleus

Fig. 41 Section of the connective tissues surrounding the gut and gut epithelium of O. <u>grenosella</u> at 120 hours after inoculation with NPV X200

P - Polyhedra
CT - Connective tissues
G - Gut epithelium

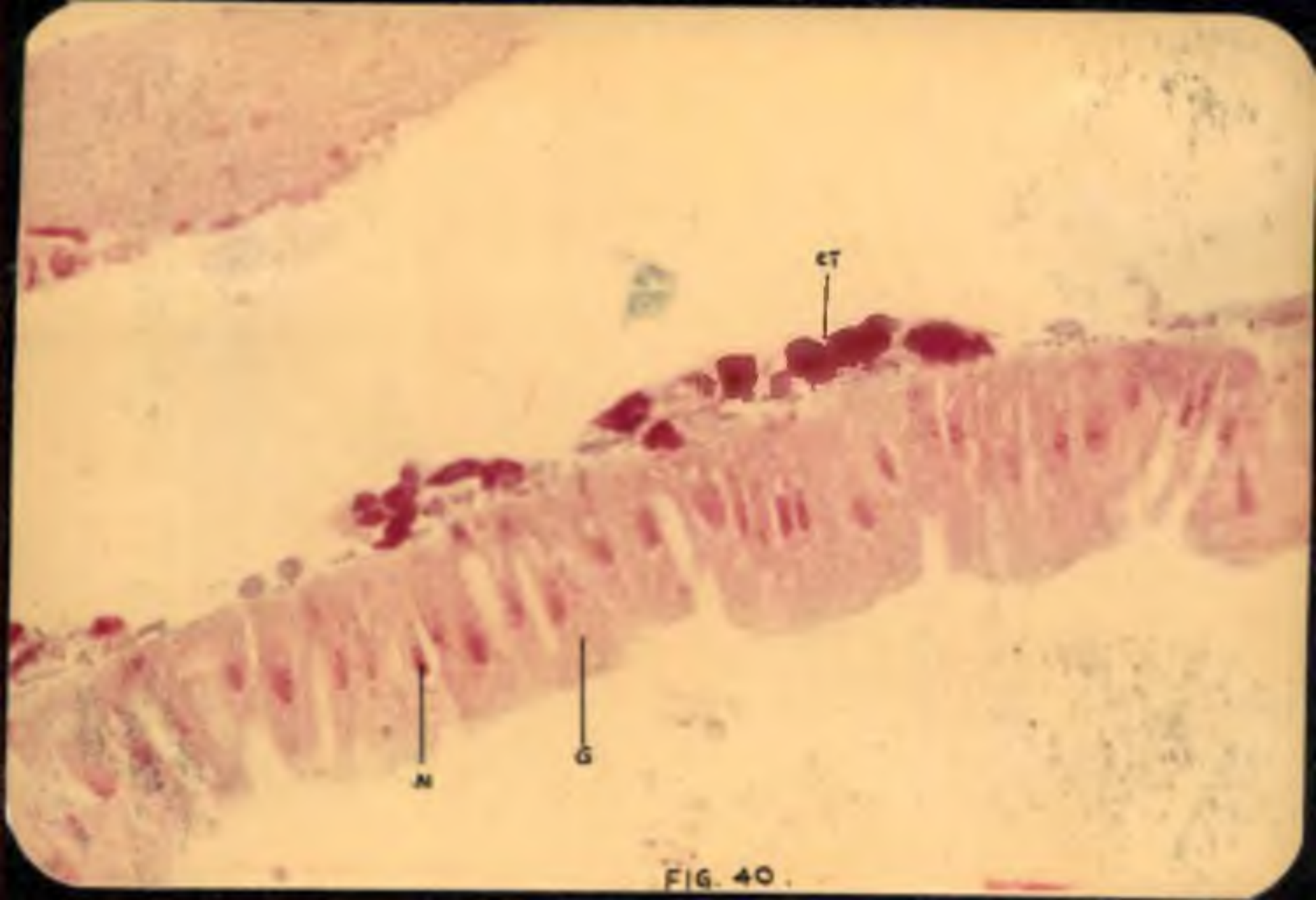


FIG. 40.

Fig. 42 Section of the connective tissues surrounding gut and gut epithelium of O. arenosella fixed at 144 hours after inoculation with NPV X500

P - Polyhedra

G - Gut epithelium

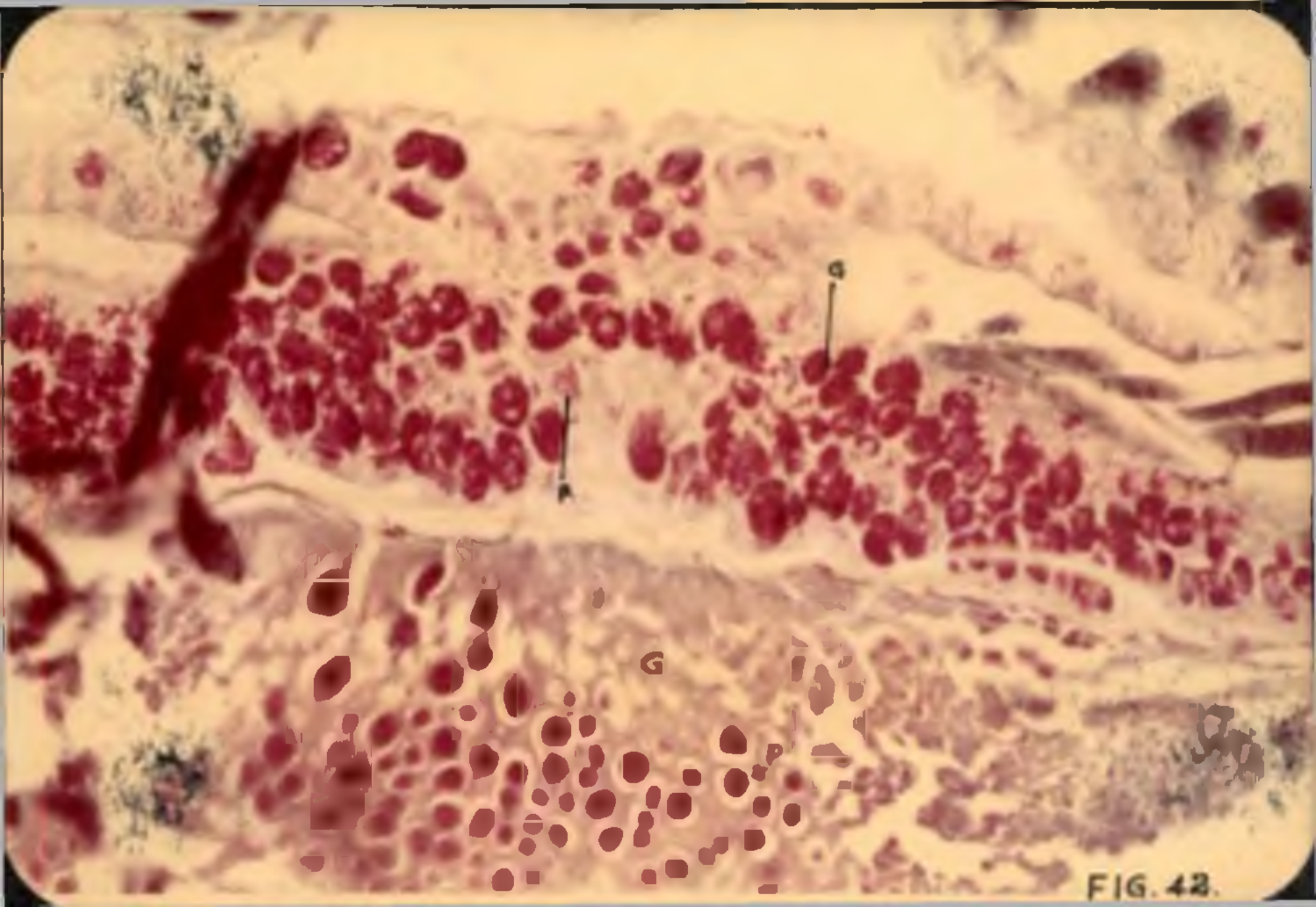


FIG. 42.

Malpighian tubules

There was no apparent difference between the Malpighian tubules of healthy and infected larvae for the first 48 hours following infection.

Seventy two hours after inoculation many nuclei were seen infected (Fig.43). The polyhedra were seen fully developed at 96 hours after inoculation (Fig. 44).

At 120 hours after inoculation the infection was seen in more number of cells (Fig.45) and by 144 hours most of the cells in the Malpighian tubules were heavily infected. Some of the cells ruptured liberating the polyhedra (Fig. 46) into the haemocoel.

Nerve ganglion

The ganglion did not show any infection at 24 and 48 hours after inoculation.

At 72 hours after inoculation commencement of infection could be seen in the neurilemma surrounding the ganglion (Fig.47). At 96 hours the polyhedral infection could be seen in ganglion cells and in neurilemma (Fig.48). At 120 hours after inoculation the infection became severe (Fig.49) and as a result of this the neurilemma

Fig. 43 Section of the Malpighian tubules of O. arenosella fixed at 72 hours after inoculation with NPV X200

MT - Malpighian tubules

N - Nucleus

Fig. 44 Section of the Malpighian tubules of O. arenosella fixed at 96 hours after inoculation with NPV X200

MT - Malpighian tubules

P - Polyhedra

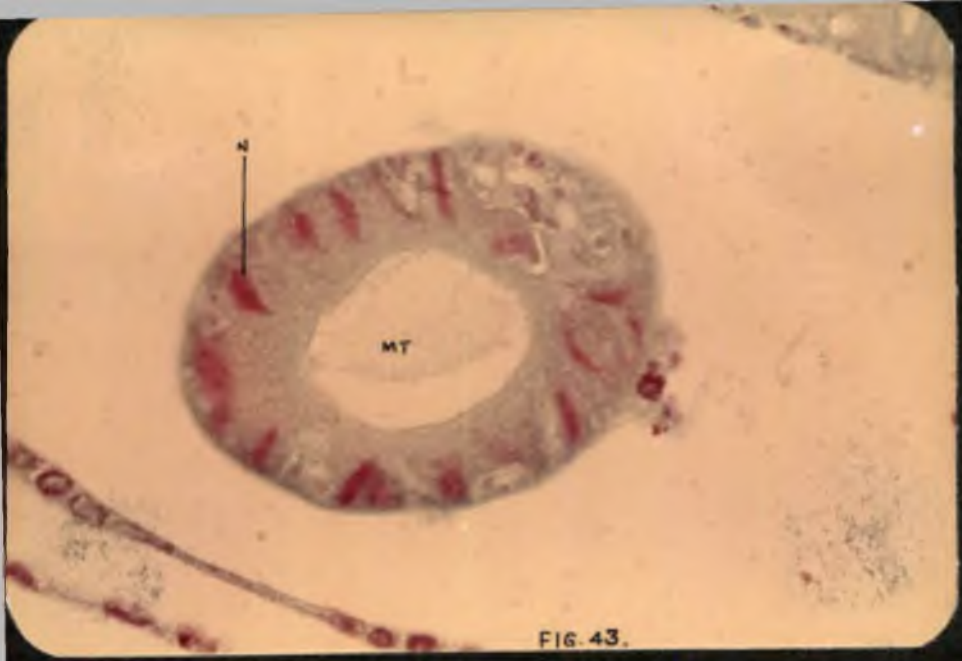


FIG. 43.

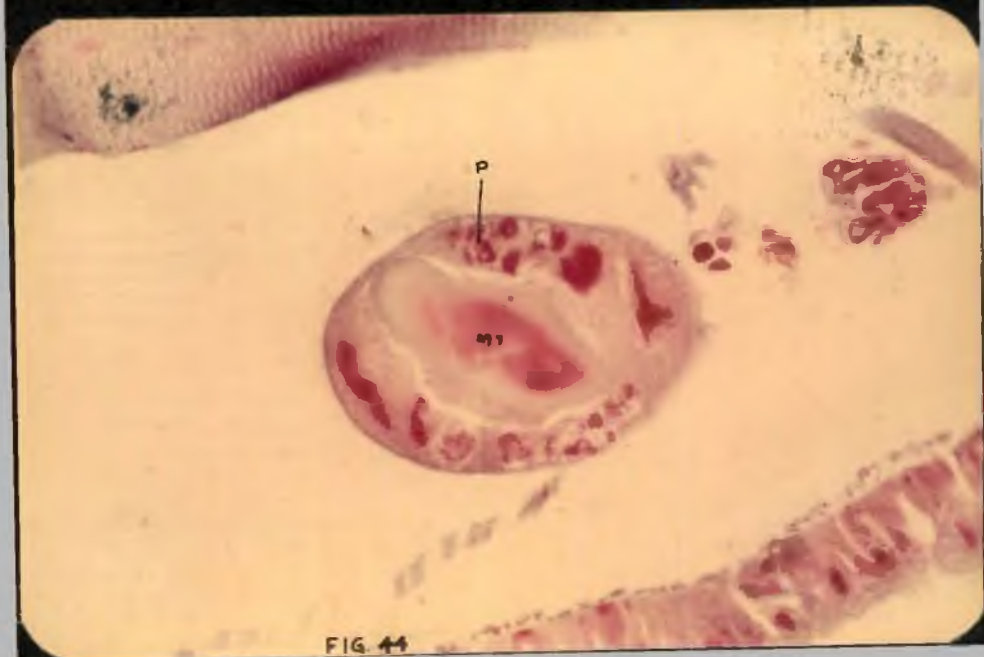


FIG. 44

Fig. 45 Section of the Malpighian tubules of *O. arenosella* fixed at 120 hours after inoculation with NPV X200

MT - Malpighian tubules

N - Enlarged nucleus

Fig. 46. Section of the Malpighian tubules of *O. arenosella* fixed at 144 hours after inoculation with NPV X200

MT - Malpighian tubules

P - Polyhedra

FIG. 45.

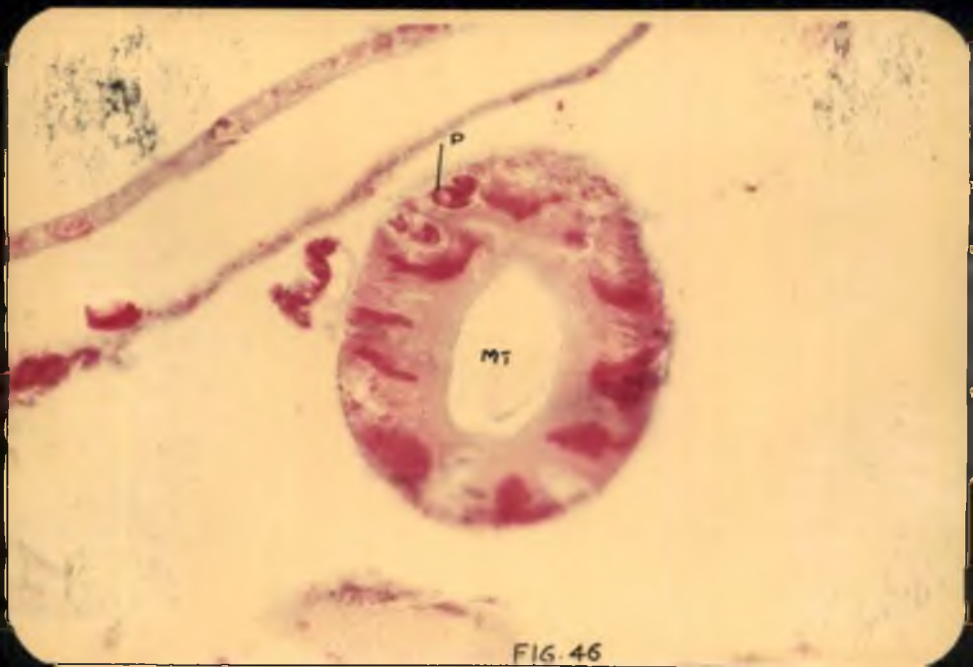


FIG. 46

Fig. 47 Section of the nerve ganglion of O. arenosella fixed at 72 hours after inoculation with NPV X200

NG - Nerve ganglion
NL - Neurilemma

Fig. 48 Section of the nerve ganglion of O.arenosella fixed at 96 hours after inoculation with NPV X200

NG - Nerve ganglion
NL - Neurilemma

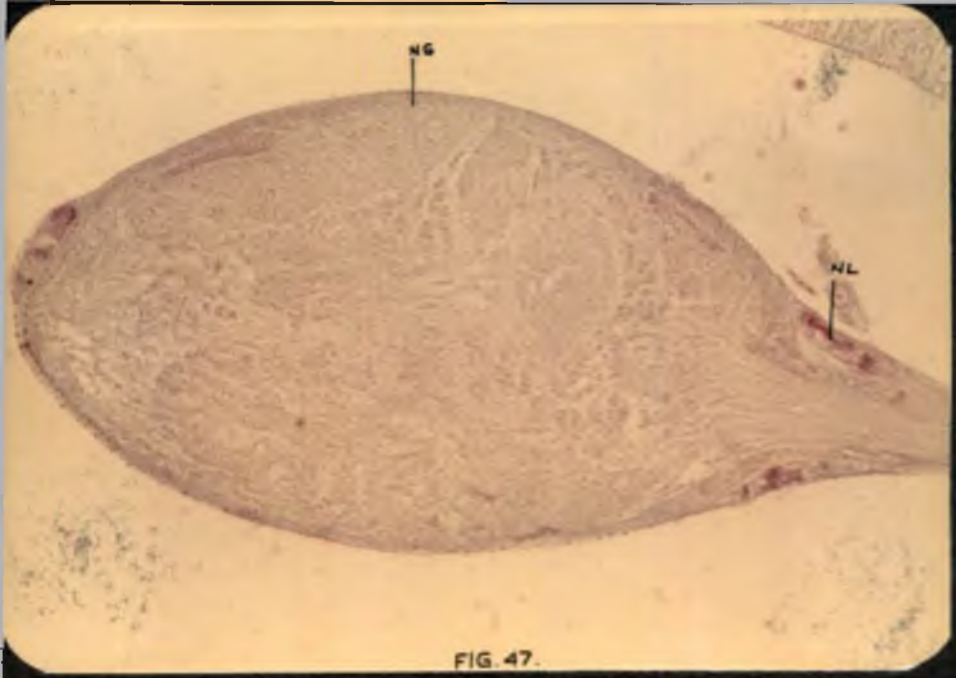


FIG. 47.

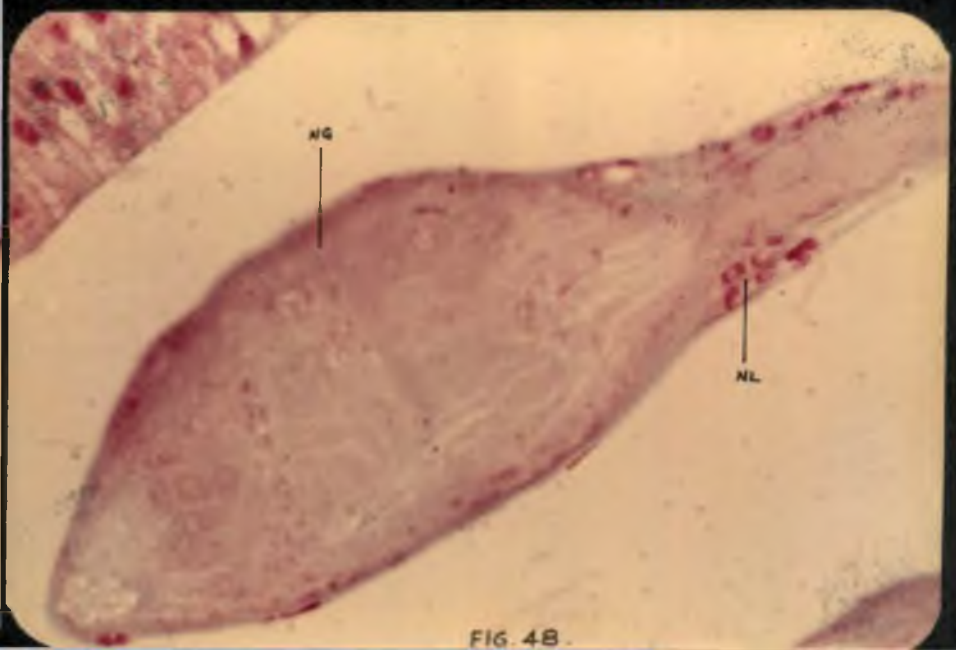


FIG. 48.

got loosened. The infection was intense in the neuropile mass (Fig.50) and neurilemma (Fig.51) at 144 hours after inoculation. Fully developed polyhedra also could be seen in the infected nuclei.

Gonads

The epithelial sheath of gonads was seen infected at 96 hours after inoculation (Fig.52). At 120 hours following infection the epithelial sheath was seen completely infected (Fig.53). At 144 hours after inoculation disintegration of cells and rupturing of the nuclei occurred leading to the liberation of polyhedra into the surrounding media (Fig.54).

4.4. Transmission through eggs

Table 1 given the per cent larval mortality, pupation and adult emergence from eggs of moths obtained from virus treated larvae. Only 1.02 per cent of larvae from surface sterilized eggs died of nuclear polyhedrosis virus while there was 43.72 per cent mortality of larvae reared from unsterilized eggs. No mortality occurred in larvae which emerged from the eggs of healthy virus free moths kept as control. But 2.56 per cent pupal mortality due to nuclear polyhedrosis virus was observed in the lot

Fig. 49 Section of nerve ganglion of O. arenosella fixed at 120 hours after inoculation with NPV X200

NG - Nerve ganglion

P - Polyhedra

Fig.50 Section of the nerve ganglion of O.arenosella fixed at 144 hours after inoculation with NPV X500

P - Polyhedra

NG - Nerve ganglion cells

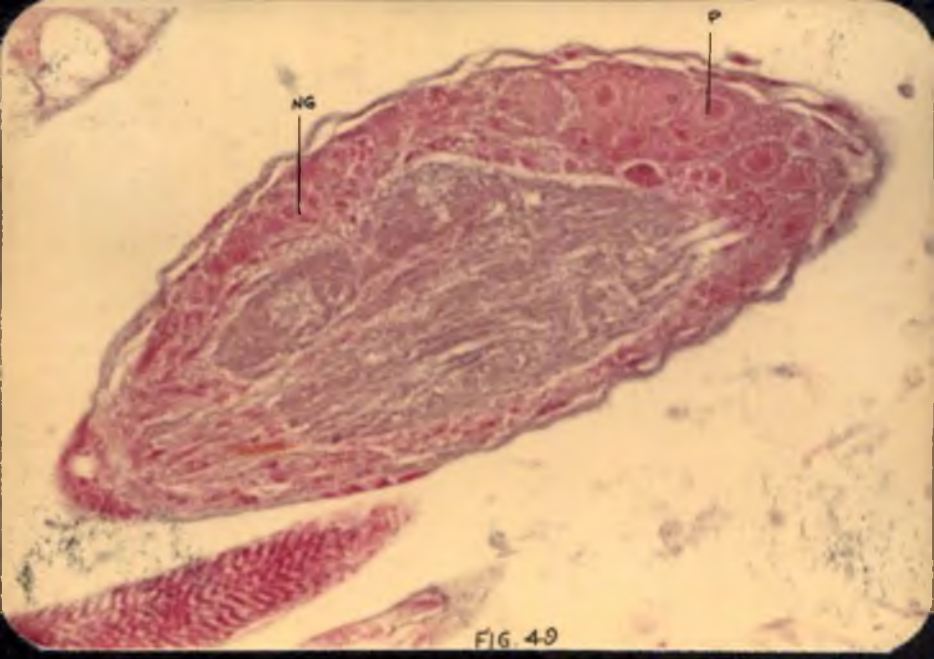


FIG. 49

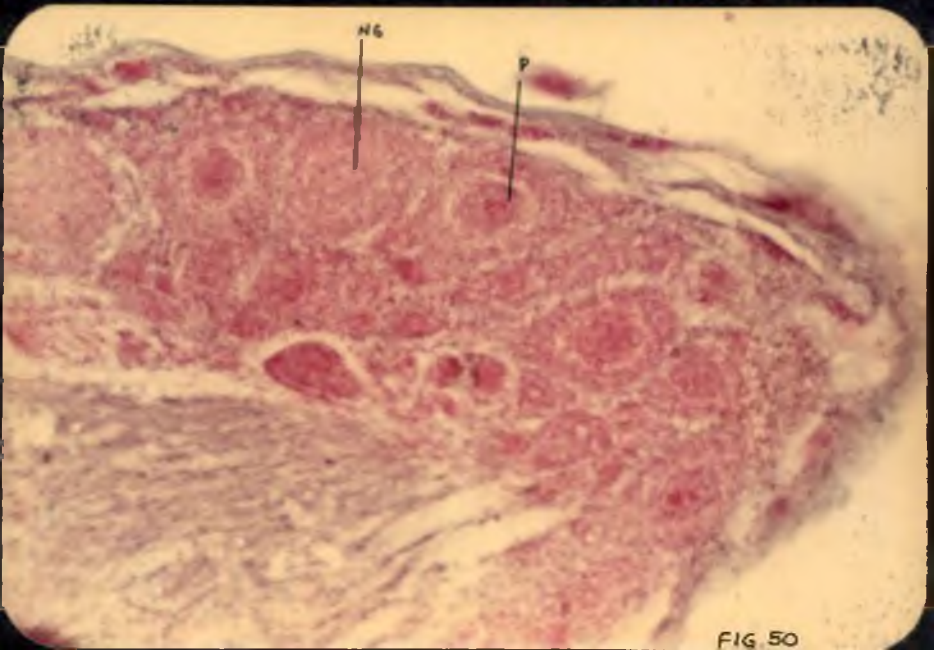


FIG. 50

Fig. 51 Section of the nerve ganglion of *D. arenosella* fixed at 144 hours after inoculation with NPV X500

NL - Neurilemma

P - Polyhedra

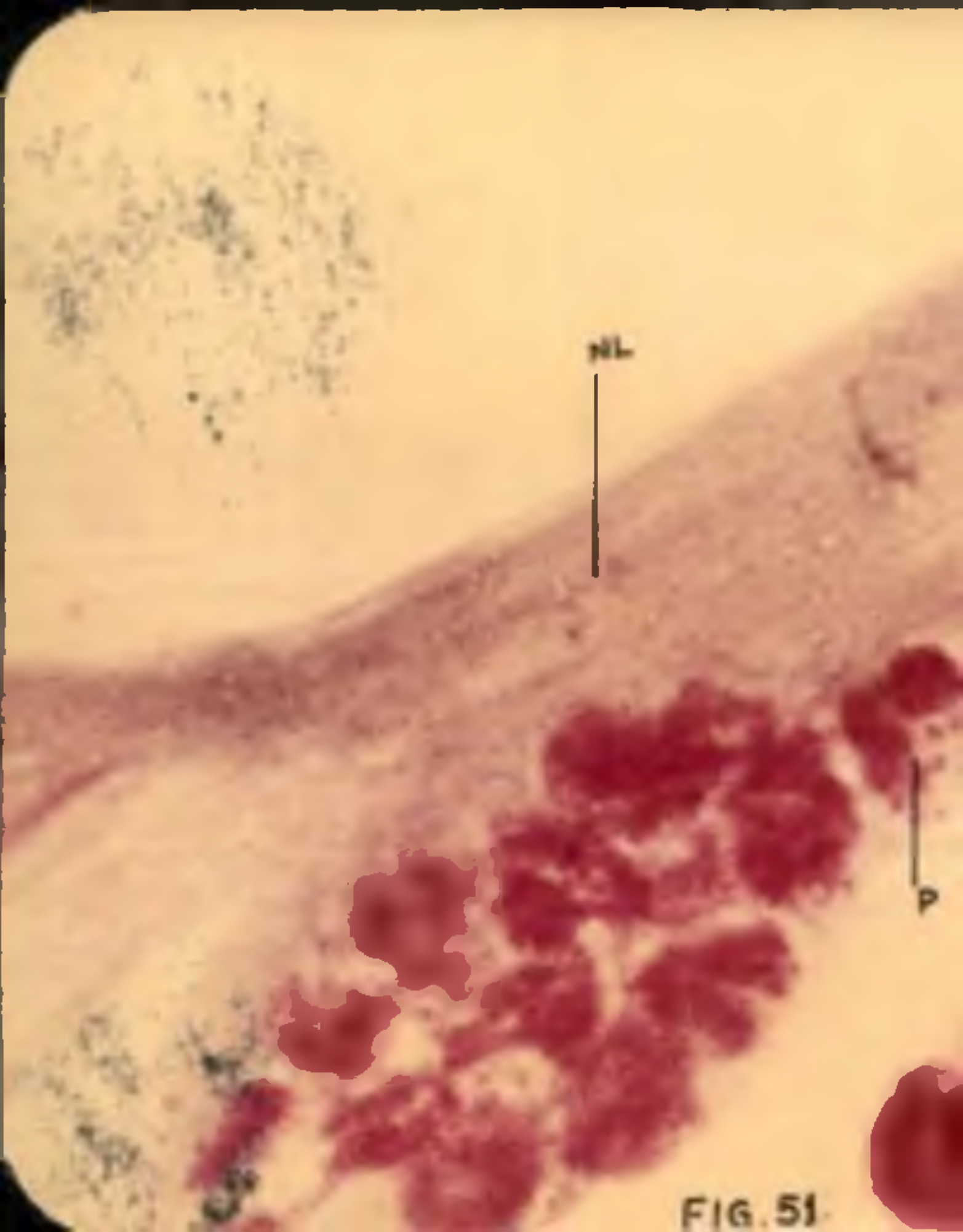


FIG. 51

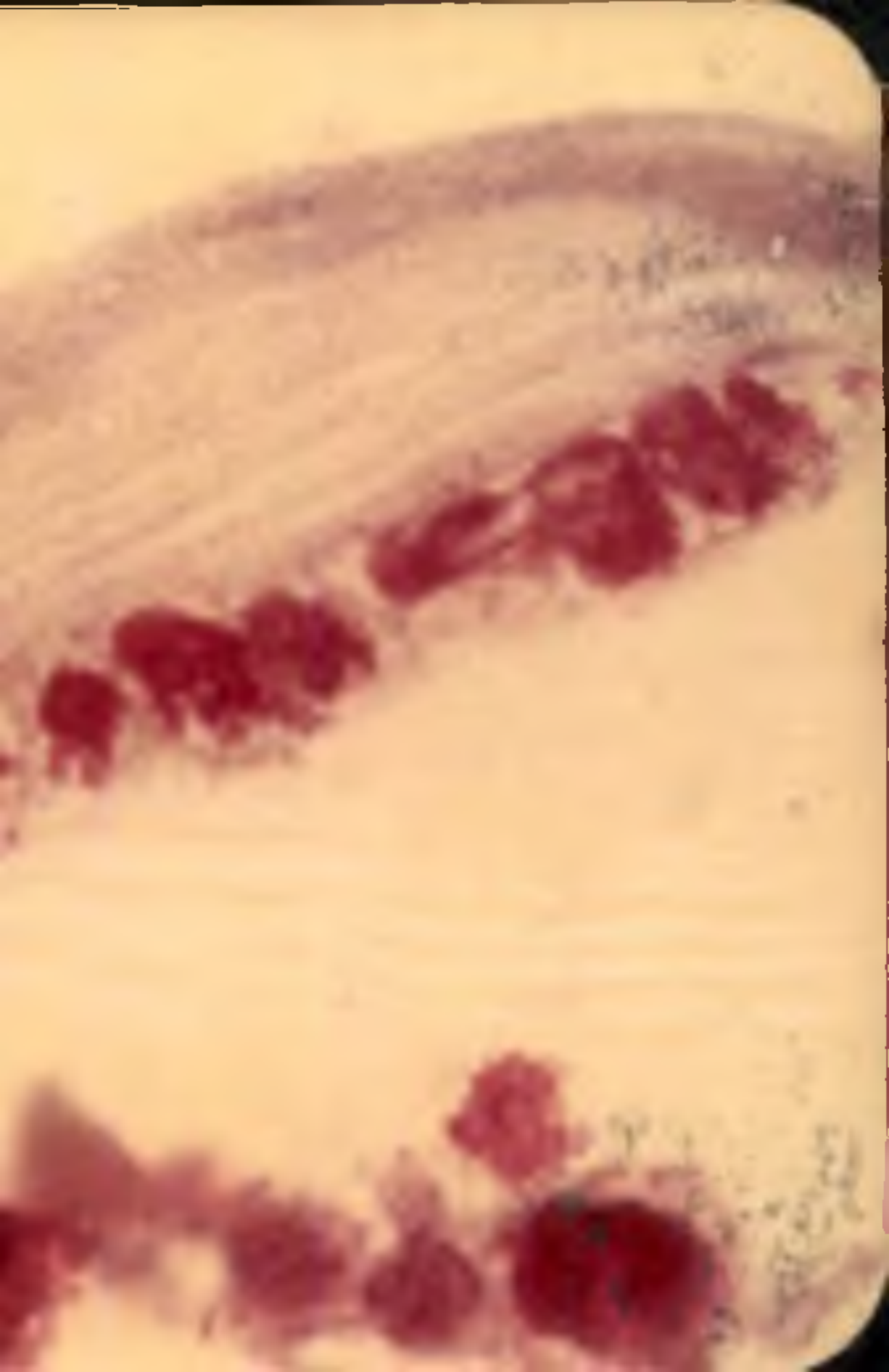


Fig. 52 Section of the gonads of O. *arenosella* fixed at 96 hours after inoculation with NPV X200

LS - Epithelial sheath of gonads

Fig. 53 Section of the gonads of O. *arenosella* fixed at 120 hours after inoculation with NPV X200

P - Polyhedra

ES - Epithelial sheath of gonads

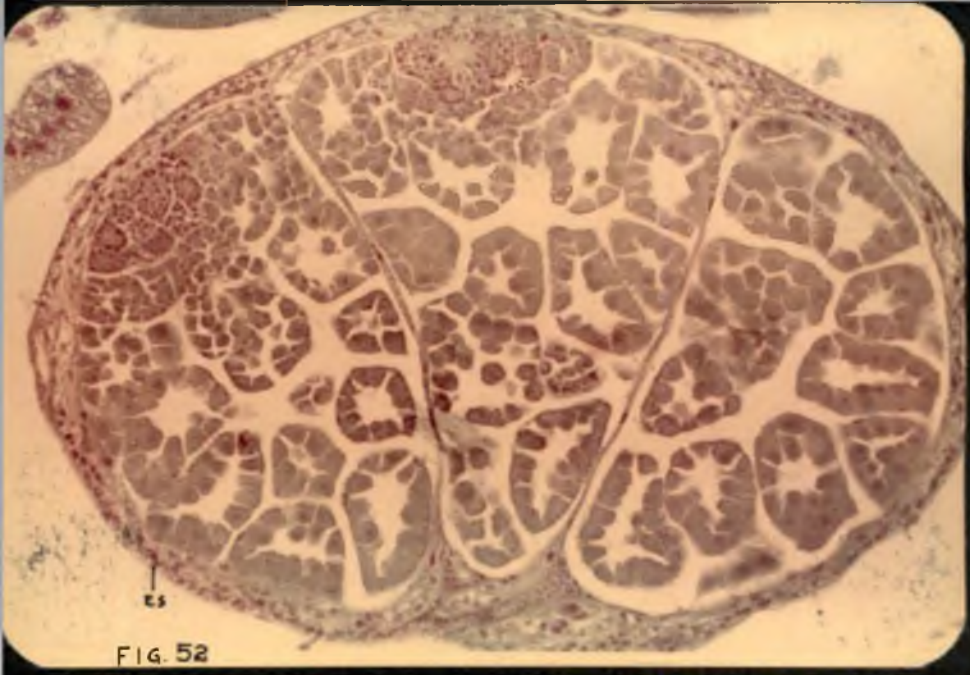


FIG. 52

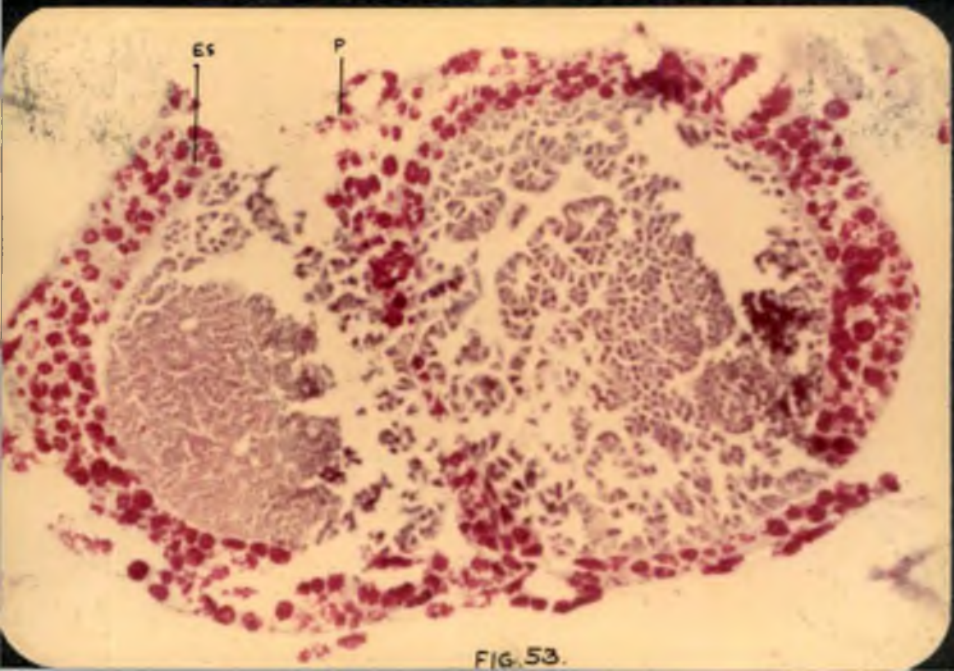


FIG. 53.

Fig. 54 Section of the gonads of *G. mellonella*
fixed at 14[illegible] hours after inoculation
with NPV X500

P = Polyhedra

ES = Epithelial sheath ruptured

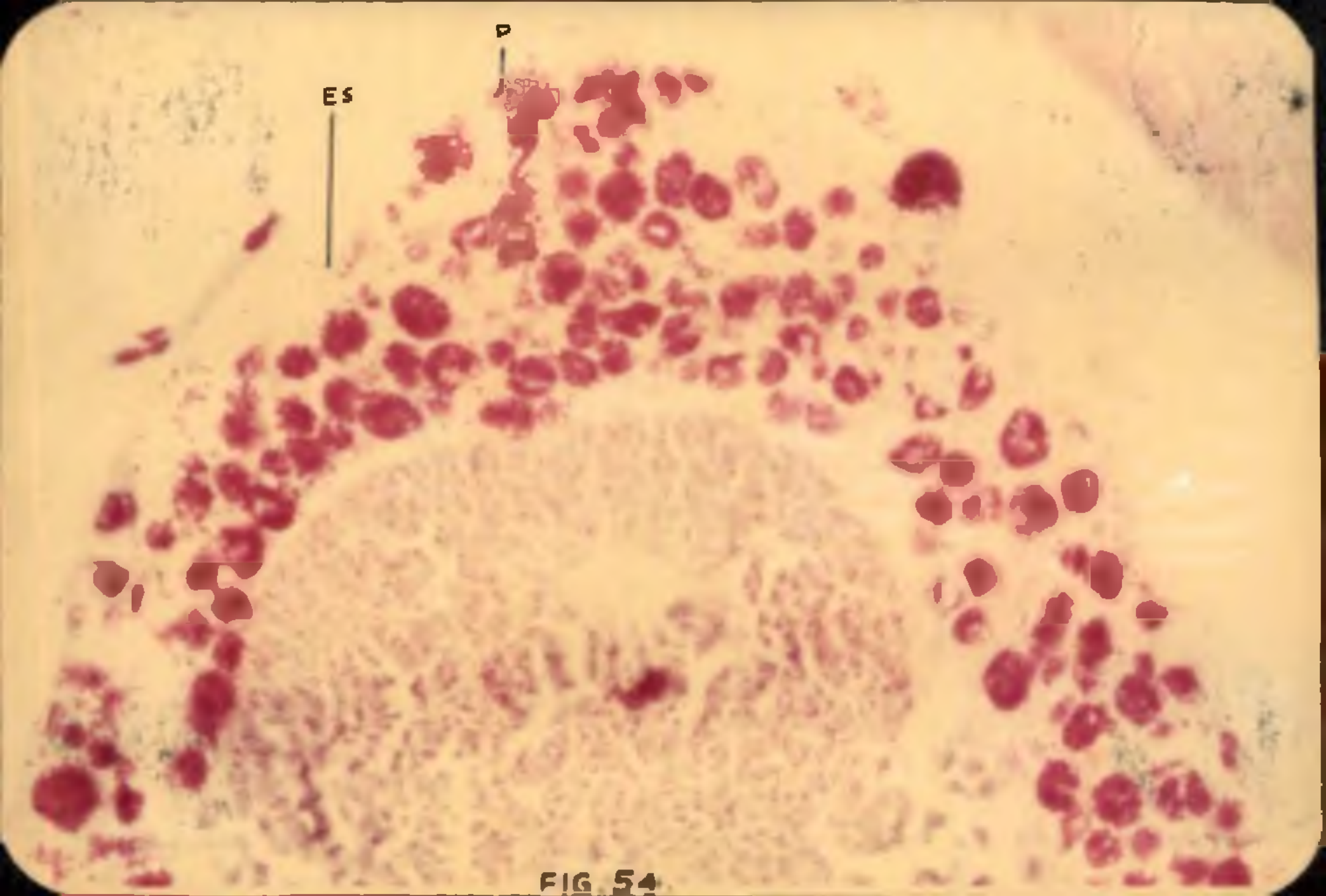


FIG. 54

Table 1. Effect of surface sterilization of the eggs obtained from moths reared out of NPV infected and uninfected larvae of O. arenosella.

Treatments	No. of eggs taken	No. of eggs hatched	Percentage of larval mortality due to		Percentage of pupal mortality due to		Percentage of adult emergence
			NPV	Other causes	NPV	Other causes	
Eggs from infected moths (surface sterilized)	200	197	1.02	1.52	Nil	Nil	97.46
Eggs from infected moths (unsterilized)	200	195	48.72	1.54	2.56	Nil	47.18
Eggs from virus free healthy moths (control)	200	195	Nil	1.54	Nil	Nil	98.46

obtained from unsterilized eggs. The percentages of adult emergence were 97.46, 47.18 and 93.46 in the lots of surface sterilized eggs, unsterilized eggs and in control respectively.

Results of feeding third instar larvae with homogenates of surface sterilized and unsterilized eggs obtained from moths which acquired the virus in the larval stages are presented in Table 2. The data showed that feeding the larvae with homogenates of surface sterilized eggs caused only 2.00 per cent mortality due to nuclear polyhedrosis virus. But when the homogenates of unsterilized eggs were fed to the larvae the mortality increased to 35.00 per cent. There was no mortality due to nuclear polyhedrosis virus among the larvae fed with homogenate of eggs obtained from healthy virus free moths. Pupal mortality due to nuclear polyhedrosis virus was not observed either in the group fed with surface sterilized egg homogenate or in the control. But 2.00 per cent pupal mortality due to nuclear polyhedrosis virus was observed in the group fed with homogenate of unsterilized eggs. The percentage of normal adult emergence were 96.00, 61.00 and 96.00 in the lots of larvae fed with homogenate of surface sterilized eggs, unsterilized eggs and eggs in control respectively.

Table 2. Effect of feeding third instar larvae of O. arenosella with homogenate of eggs laid by moths reared out of larvae fed with NPV and of eggs laid by uninfected moths.

Treatments	No. of larvae fed	Percentage of larval mortality due to		Percentage of pupal mortality due to		Percentage of adult emergence
		NPV	other causes	NPV	Other causes	
Homogenate of surface sterilised eggs	100	2.00	2.00	Nil	Nil	96.00
Homogenate of unsterilized eggs	100	35.00	2.00	2.00	Nil	61.00
Homogenate of eggs from healthy virus free moths (control)	100	Nil	2.00	Nil	2.00	96.00

4.4.1. Transmission through virus-fed moths

Table 3 presents the data on larval mortality, pupation and adult emergence obtained from the eggs of virus fed adults kept with and without surface sterilization. It can be seen that there was 12.24 per cent larval mortality due to nuclear polyhedrosis virus in the progeny obtained from unsterilized eggs while there was no mortality due to nuclear polyhedrosis virus among the larvae emerging from sterilized eggs and in the eggs in control group. Progeny from unsterilized eggs of virus fed moths recorded 2.04 per cent pupal mortality due to nuclear polyhedrosis virus but no pupal mortality was observed in the progeny from sterilized eggs and the eggs in control. The percentages of adult emergence were 98.97, 84.70 and 98.99 in progenies reared from eggs obtained from virus fed moths of surface sterilized and unsterilized and in control respectively.

4.4.2. Transmission through contamination of female genitalia

The data on disease incidence in the progeny when the female genitalia were contaminated with the virus are furnished in Table 4. When the eggs obtained from conta-

Table 3. Incidence of NPV in the progeny of moths of *O. arenosella* fed with the virus

Treatments	No. of eggs taken	No. of eggs hatched	Percentage of larval mortality due to		Percentage of pupal mortality due to		Percentage of adult emergence
			NPV	Other causes	NPV	Other causes	
Eggs from virus fed moths sterilized	100	97	Nil	1.03	Nil	Nil	98.97
Unsterilized	100	98	12.24	1.02	2.04	Nil	84.70
Eggs from healthy virus free moths (control)	100	99	Nil	1.01	Nil	Nil	98.99

Table 4. Incidence of NPV in the progeny of *O. sesamoella* when the moths were contaminated with the virus on their external genitalia

Treatments	No. of eggs taken	No. of eggs hatched	Percentage of larval mortality due to		Percentage of Pupal mortality due to		Percentage of adult emergence
			NPV	Other causes	NPV	Other causes	
Eggs from virus contaminated moths surface sterilized	150	147	Nil	2.72	Nil	Nil	97.28
Unsterilized	150	148	41.89	3.38	Nil	Nil	54.73
Eggs from healthy virus free moths (control)	150	148	Nil	2.70	Nil	Nil	97.30

minated moths were left unsterilized 41.89 per cent of the emerging larvae died due to nuclear polyhedrosis virus infection. But no death due to viral infection was observed when the eggs were surface sterilized. There was no disease incidence in control also. The percentages of adult emergence from surface sterilized and unsterilized eggs laid by virus contaminated moths and those in control lot were 97.28, 54.73 and 97.30 respectively.

4.5. Assessing cross-infectivity of the virus to the larvae of insects belonging to different families Lepidoptera

Data presented in Table 5 showed that the larvae of Oplsina arenosella, Spodoptera litura and Spodoptera mauritia were highly susceptible to nuclear polyhedrosis virus of O. arenosella recording 100, 80 and 79 per cent mortality respectively. The larvae of Anedevidia poponis, Diacrisia obliqua and Pericallia ricini were moderately susceptible to the virus, the mortality recorded being 70, 69 and 63 per cent respectively. The larvae of Orthaga exvinacease, Euproctis fraterna, Sylepta derogata, Margaronia indica and Cnaphalocrocis medinalis were not

Table 5. Infectivity of NPV of *O. arenosella* to third instar larvae of other locally available Lepidoptera which were amenable to laboratory rearing

Insects tested	Percentage of larval mortality due to		Incubation period in days		Infectivity	Larval period in days	Quantity of polyhedra/ml of body fluid
	NPV	Other causes	Range	Mean			
1. *Opisina arenosella*	100	Nil	3 to 6	4.01	+ve	30 to 40	2.17×10^7
2. *Spodoptera litura*	80	Nil	2 to 6	3.09	+ve	20 to 25	2.22×10^7
3. *Spodoptera mauritia*	79	Nil	2 to 6	3.26	+ve	16 to 20	2.20×10^7
4. *Anadevidia peponis*	70	Nil	3 to 7	3.46	+ve	25 to 30	2.02×10^6
5. *Pericallia ricini*	63	Nil	3 to 8	3.18	+ve	20 to 25	2.09×10^6
6. *Diacrisia obliqua*	69	Nil	4 to 9	4.22	+ve	30 to 40	1.88×10^6
7. *Orthaga exvinacease*	Nil	Nil	-	-	-ve	25 to 30	
8. *Euproctis fraterna*	Nil	3	-	-	-ve	25 to 30	
9. *Sylepta derogata*	Nil	Nil	-	-	-ve	18 to 22	
10. *Margaronia indica*	Nil	Nil	-	-	-ve	15 to 20	
11. *Cnaphalocrocis medinalis*	Nil	4	-	-	-ve	23 to 25	

susceptible to nuclear polyhedrosis virus of *O. arenosella* and the treated larvae pupated normally and adults emerged except for those which died due to other causes.

The mean incubation periods of nuclear polyhedrosis virus in *O. arenosella*, *S. litura*, *S. mauritia*, *A. pernonis*, *P. ricini* and *D. obliqua* were 4.01, 3.09, 3.26, 3.46, 3.18 and 4.22 days respectively. The numbers of polyhedra per millilitre body fluid were 2.17×10^7, 2.22×10^7, 2.20×10^7, 2.02×10^6, 2.09×10^6 and 1.88×10^6 in *O. arenosella*, *S. litura*, *S. mauritia*, *A. pernonis*, *P. ricini* and *D. obliqua* respectively. The pathogen multiplied in all the hosts were equally effective and as virulent as the original stock obtained from *O. arenosella* and were cross infective to each other.

4.6. Evaluating the efficacy of the virus against *O. arenosella*

4.6.1. Bioassay of the virus

The data relating to the experiments are presented in Tables 6 and 7, appendix I and illustrated in Fig.55.

The mortality due to viral infection ranged from 100 to 38 per cent for the first instar larvae for the doses

Table 6 Effect of different doses of NPV on the different larval instars of O. *armigella*

Dose	Percentage of larval mortality	Time taken for death (range)	Mean time taken for death (days)	LT_{50} *	Fiducial limits	Regression equation	Hetero genesity χ^2
1	2	3	4	5	6	7	8
FIRST INSTAR							
1.96 x 10^5 P.Bs per ml	100	3 to 5	3.84	3.08	2.81 3.14	Y = 6.176x + 2.112	3.15
1.96 x 10^4 "	100	2 to 6	4.00	3.26	3.13 3.42	Y = 5.436x + 2.205	3.85
1.96 x 10^3 "	92	3 to 7	5.09	5.01	4.42 5.61	Y = 4.613x + 1.915	2.68
1.96 x 10^2 "	78	3 to 7	4.15	7.40	6.98 7.93	Y = 4.197x + 1.354	1.15
1.96 x 10 "	52	3 to 9	6.58	8.70	8.10 9.20	Y = 5.553x + 0.682	1.44
1.96	38	3 to 9	6.76	-			-
SECOND INSTAR							
1.96 x 10^7 P.Bs per ml	100	3 to 7	5.06	4.28	4.12 4.47	Y = 6.001x + 1.205	1.37
1.96 x 10^6 "	100	3 to 7	5.10	4.33	4.19 4.49	Y = 7.139x + 0.455	6.76
1.96 x 10^5 "	96	3 to 7	5.15	4.51	4.32 4.69	Y = 7.276x + 0.237	5.34
1.96 x 10^4 "	84	3 to 7	6.45	6.91	4.24 3.19	Y = 5.845x + 0.943	1.88
1.96 x 10^3 "	76	3 to 8	6.66	6.24	5.34 9.13	Y = 3.641x + 2.371	2.22
1.96 x 10^2 "	48	4 to 9	7.50		-	-	
THIRD INSTAR							
1.96 x 10^7 PIBs per ml	100	3 to 7	5.18	4.22	4.19 4.56	Y = 5.009x + 1.812	1.54
1.96 x 10^6 "	96	3 to 7	6.19	4.42	4.27 4.60	Y = 6.653x + 0.707	6.96
1.96 x 10^5 "	80	4 to 8	7.15	6.26	5.87 6.79	Y = 4.542x + 1.381	7.61
1.96 x 10^4 "	72	4 to 8	7.64	7.61	7.35 9.20	Y = 5.716x - 0.267	6.43
1.96 x 10^3 "	52	5 to 10	8.85	9.64	8.40 11.59	Y = 5.553x + 0.792	1.44
1.96 x 10^2 "	40	6 to 11	9.50	-			
FOURTH INSTAR							
1.96 x 10^7 PIBs per ml	86	4 to 9	6.98	6.91	6.66 7.21	Y = 6.572x - 0.517	2.11
1.96 x 10^6 "	84	4 to 10	7.07	7.22	6.46 7.39	Y = 13.510x - 9.901	1.91
1.96 x 10^5 "	76	4 to 11	7.58	8.14	7.82 8.52	Y = 5.134x + 0.324	7.56
1.96 x 10^4 "	62	4 to 11	8.32	10.38	9.85 11.02	Y = 4.362x + 0.567	1.61
1.96 x 10^3	46	5 to 12	10.57	-	-	-	-
1.96 x 10^2 "	38	7 to 13	11.47		-		-
FIFTH INSTAR							
1.96 x 10^7 PIBs per ml	62	4 to 11	9.19	9.77	9.07 10.16	Y = 9.684x + 0.616	2.51
1.96 x 10^6 "	56	4 to 11	9.29	10.96	10.79 13.00	Y = 3.078x + 0.501	10.01
1.96 x 10^5 "	52	5 to 12	10.57	11.99	10.96 12.30	Y = 9.718x - 4.503	4.20
1.96 x 10^4 "	42	5 to 12	11.09	-	-	-	
1.96 x 10^3	30	7 to 14	13.00	-	-		-
1.96 x 10^2 "	14	9 to 15	13.57	-	-		

* LT_{50} Time required to give 50 per cent mortality of larvae

In all the cases the data were homogenous at P 0.05

Table 7. Relative susceptibility of different larval instars of O. *prenoselia* to nuclear polyhedrosis

Larval instar	Hetero-genity** x^2	Regression equation	LC_{50}* (PIBs/ml)	Fiducial limits (PIBs/ml)	Order of relative efficacy (ORE)
First	2.66	Y = 0.6059x + 4.4277	9.247	4.730 15.010	-
Second	3.17	Y = 0.5902x + 3.6114	225.424	92.045 456.801	24.38
Third	6.47	Y = 0.4978x + 3.4977	1044.961	507.692 1934.639	113.01
Fourth	1.35	Y = 0.3076x + 3.9693	2242.332	768.776 5367.845	242.49
Fifth	9.02	Y = 0.2886x + 3.4047	33775.361	14821.768 96205.522	3652.58

*LC_{50} = Concentration required to give 50 per cent mortality to test larvae

** = In all the cases the data were homogenous at P = 0.05

Fig. 55 Response of different larval instars of Oxisina arenosella to varying doses of nuclear polyhedrosis virus.

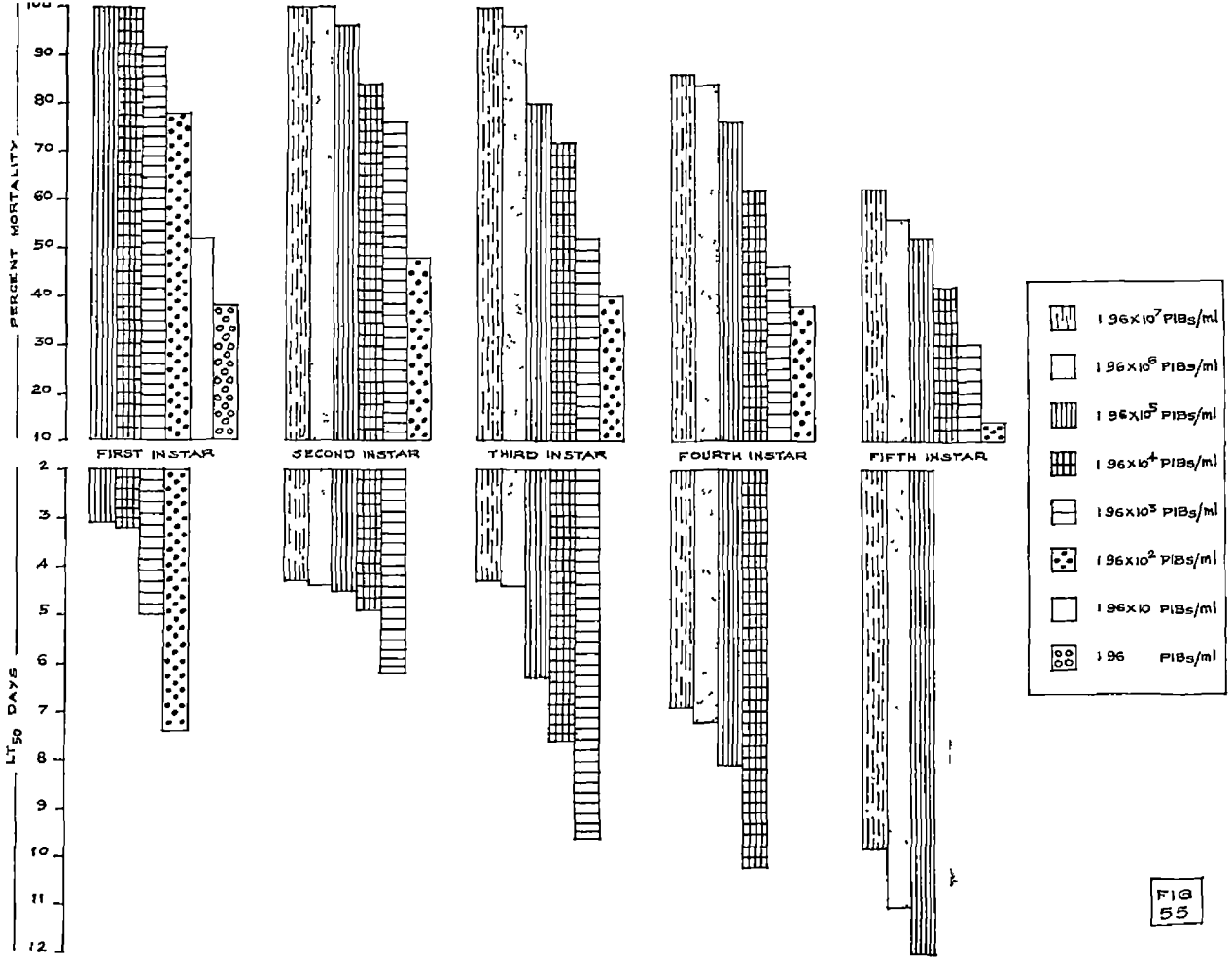

PERCENT MORTALITY
100
90
80
70
60
50
40
30
20
10
FIRST INSTAR
SECOND INSTAR
THIRD INSTAR
FOURTH INSTAR
FIFTH INSTAR
LT50 DAYS
2
3
4
5
6
7
8
9
10
11
12
1.96x10^7 PIBs/ml
1.96x10^6 PIBs/ml
1.96x10^5 PIBs/ml
1.96x10^4 PIBs/ml
1.96x10^3 PIBs/ml
1.96x10^2 PIBs/ml
1.96x10 PIBs/ml
1.96 PIBs/ml

FIG 55

ranging from 1.96×10^5 PIBs/ml to 1.96 PIBs/ml. The time taken for death ranged from 2 to 9 days and the mean time taken for death ranged from 3.84 to 6.78 days. For the different doses tried, the LT_{50} ranged from 3.08 to 8.70 days and the fiducial limits were also not seen high. The LT_{50} values for the concentration 1.96 PIBs/ml could not be calculated since the mortality recorded for this concentration was less than 50 per cent.

The per cent mortality of second instar larvae ranged from 100 to 48 for the doses ranging from 1.96×10^6 PIBs/ml to 1.96×10^2 PIBs/ml. The time taken for death ranged from 3 to 9 days and the mean time taken for death ranged from 5.06 to 7.50 days. The LT_{50} values for the different doses ranged from 4.28 to 6.24 days. At the lowest concentration of the virus tested (1.96×10^2 PIBs/ml) the LT_{50} could not be calculated as the mortality recorded was less than 50 per cent.

Mortalities ranging from 100 to 40 per cent were observed for the third instar larvae for the doses ranging from 1.96×10^7 PIBs/ml to 1.96×10^2 PIBs/ml. The time taken for death ranged from 3 to 11 days and the mean time taken for death ranged from 5.18 to 9.50 days. For the different doses tried the LT_{50} ranged from 4.32 to 9.64 days. The LT_{50} value for the concentration 1.96×10^2PIBs/ml

could not be calculated as the mortality recorded at this dose was below 50 per cent.

The per cent mortality of fourth instar larvae ranged from 86 to 38 for the doses ranging from 1.96×10^7 PIBs/ml to 1.96×10^2 PIBs/ml. The time taken for death ranged from 4 to 13 days and the mean time taken for death ranged from 6.98 to 11.47 days. The LT_{50} values for different doses ranged from 6.91 to 10.38 days. The LT_{50} values for the concentrations 1.96×10^3 PIBs/ml and 1.96×10^2 PIBs/ml could not be calculated as the maximum cumulative mortalities recorded were below 50 per cent.

The per cent mortality of fifth instar larvae ranged from 62 to 14 for the doses ranging from 1.96×10^7 PIBs/ml to 1.96×10^2 PIBs/ml. The time taken for death ranged from 4 to 15 days and the mean values ranged from 8.19 to 13.57 days. For different doses the LT_{50} values ranged from 9.77 to 11.99 days. The LT_{50} values for the concentrations 1.96×10^4, 1.96×10^3 and 1.96×10^2 PIBs/ml could not be calculated as the maximum cumulative mortalities recorded in the treatments were below 50 per cent.

The LC_{50} values of nuclear polyhedrosis virus to first, second, third, fourth and fifth instar larvae were

9.247, 225.424, 1044.961, 2242.332 and 33775.361 PIBs/ml respectively. Taking the LC_{50} to the first instar as standard the concentration required to cause 50 per cent mortality of second, third, fourth and fifth instar larvae were calculated to be 24.38, 113.01, 242.49 and 3652.58 times higher respectively (Table 7).

4.6.2. Persistence of the virus on coconut foliage exposed to weathering

The response of third instar larvae of O.arenosella to the nuclear polyhedrosis virus exposed to weathering for varying periods on coconut foliage is shown in Table 8, appendix 1 and Fig.56.

The larval mortality due to nuclear polyhedrosis virus was reduced from 100 per cent at zero hour to zero level at 168 hour of exposure. The mortality was reduced below 50 per cent at 84 hour of weathering and the virus retained only 10 per cent of its infectivity at 156 hour of exposure. The virus was completely inactivated at 168 hour of weathering. The mean time taken for death ranged from 4.50 days at zero hour to 27.13 days at 156 hour. The LT_{50} values ranged from 4.54 days so for the virus unexposed to weathering, to 13.80 days for the lot

Table 8. Effect of feeding third instar larvae of O. arenosella on coconut leaves treated with polyhedral suspension (1.96 x 10^7 PIBs/ml) subjected to weathering for varying periods.

	Period of exposure to weathering (In hours)														
	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
Per centage of larval mortality	100 (90)*	90 (84.26)	83.33 (65.88)	76 67 (61.14)	66.67 (54.76)	60 00 (50 77)	53.33 (46.89)	50.00 (45 00)	43.33 (41.15)	40.00 (39.23	3c.63 (3?.23)	33 33 (3c.24)	30.00 (33.21)	10.00 (18.44)	0.00
Time taken for death (range)	3-c	3-9	3-9	4-11	4-12	c-13	6-14	7-18	10-19	12-20	15-22	20-25	22-27	23-33	..
Mean time taken for death (days)	4.50	5.85	6 52	7 82	10.00	10.67	12 95	13.87	16.24	17.75	20.77	23.12	25.12	27.13	
LT_{50} (days)	4.54	5 54	7 02	8.27	10.23	2 30	13 80	.	..	..	..	.		..	.
Fiducial limits	4.34 4.79	5 32 5.81	6 67 7.54	8 12 8.47	10.62 12.88	1. 48 12.46	13.48 15.85	..	..	..	..	..	.	.	..
Hetero-geneity X^2 **	3.09	2.12	10.68	3.64	2.07	2.09	3 04		.	..	.	..	..	..	.
Regression equations	A	B	C	D	E	F	G	..	..	..	.	.			.

A = Y =8 2?0x-0 444 B - Y 6.815x-0 071 C = Y = 4 32?x+1 466 D = Y =8.842x-1 344

E = Y =3 30?x+1 486 F = Y = 2 020x-1 5?0 G = Y = 5.129x-0.999

* Figures in the parentheses are values after angular transformations

** The data were homogenous at P = 0.05

Fig. 56. Effect of weathering on the viral activity of nuclear polyhedrosis virus of Oiosina aronosella applied on coconut foliage.

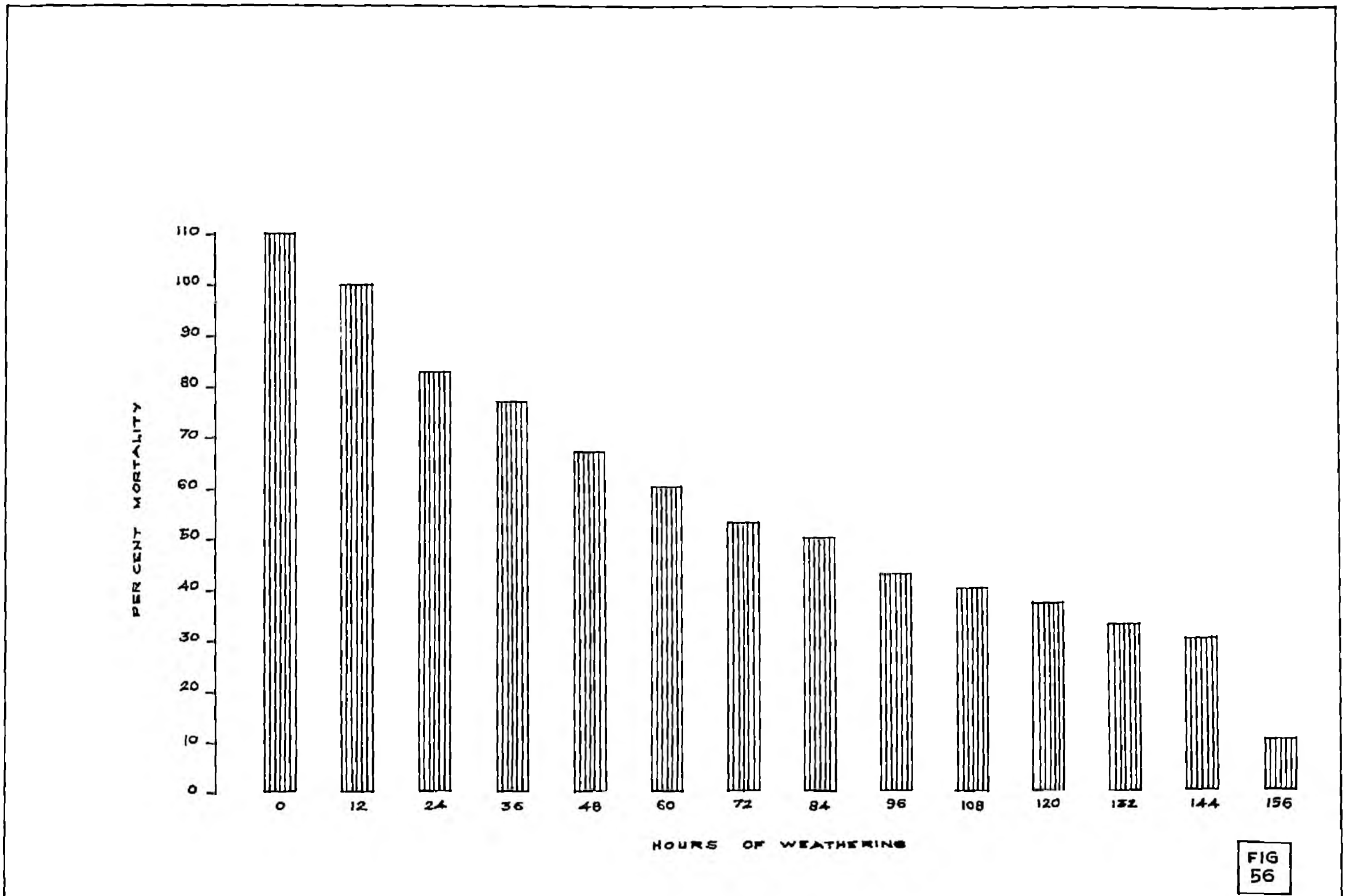

PER CENT MORTALITY
110
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
HOURS OF WEATHERING

FIG 56

exposed to weathering for 72 hours. The LT_{50} values for the virus exposed to weathering for more than 72 hours could not be calculated as the mortalities observed were below 50 per cent.

The half life of the virus (Table 12) on coconut foliage exposed to weathering was estimated as 83.25 hours.

4.7.1. Effect of exposure of the polyhedra to a constant temperature (35°C) for varying periods

The data relating to the experiment and the results of statistical analysis are presented in Table 9, appendix 1 and Fig.57.

It is seen that the larval mortality due to nuclear polyhedrosis virus was reduced from 100 per cent for the unexposed virus to zero level at 156 hour of exposure. The virus exposed to heat treatment for 84 hours retained infectivity to cause 56.69 per cent mortality of the larvae but further exposure gradually reduced the mortality and it reached to 13.33 per cent at 144 hour of exposure. The mean time taken for death was also prolonged from 4.70 days at zero hour of exposure to 13.75 days at 144 hour of exposure.

Table 9. Effect of continuous exposure of NPV of O. argentella to a constant temperature of 35°C for varying periods

	Period of exposure (hours)													
	0	12	24	36	48	60	72	84	96	108	120	132	144	156
Percentage of larval mortality	100 *(90)	96.67 (79.53)	90.67 (68.61)	80.00 (63.44)	76.67 (61.14)	66.67 (54.76)	60.00 (50.77)	56.67 (48.85)	46.67 (43.11)	43.33 (41.15)	33.33 (35.24)	16.67 (24.12)	13.33 (21.39)	
Time taken for death (range)	3-7	3-7	3-7	4-8	5-9	6-11	6-11	7-12	7-12	8-13	9-14	10-14	12-15	.
Mean time taken for death (days)	4.70	4.76	5.15	5.79	7.09	9.40	9.61	10.52	10.64	11.23	12.10	12.60	13.75	.
LT_{50} (days)	3.89	3.99	5.05	6.01	7.29	10.45	10.89	11.72	..	.	..	..	..	
Fiducial limits	3.64 4.13	3.76 4.40	4.67 5.63	5.39 6.21	6.88 7.90	10.39 13.24	10.08 12.30	11.19 12.41	..	..	..		.	..
Heterogenity **	3.55	1.60	1.62	6.44	4.03	9.25	3.92	1.92						
Regression equation	A	B	C	D	E	F	G	H						

A = Y = 5.712x + 1.631 B = Y = 4.796x + 2.079 C = Y = 4.172x + 2.064 D = Y = 4.755x + 1.389

E = Y = 5.309x + 0.367 F = Y = 5.673x - 1.126 G = Y = 5.670x - 1.017 H = Y = 7.046x - 2.537

* Figures in the parenthesis are values after angular transformations

** The data were homogenous at P = 0.05

Fig. 57. Effect of continuous exposure of nuclear polyhedrosis virus of Opisina arenosella to a constant temperature (35°C)

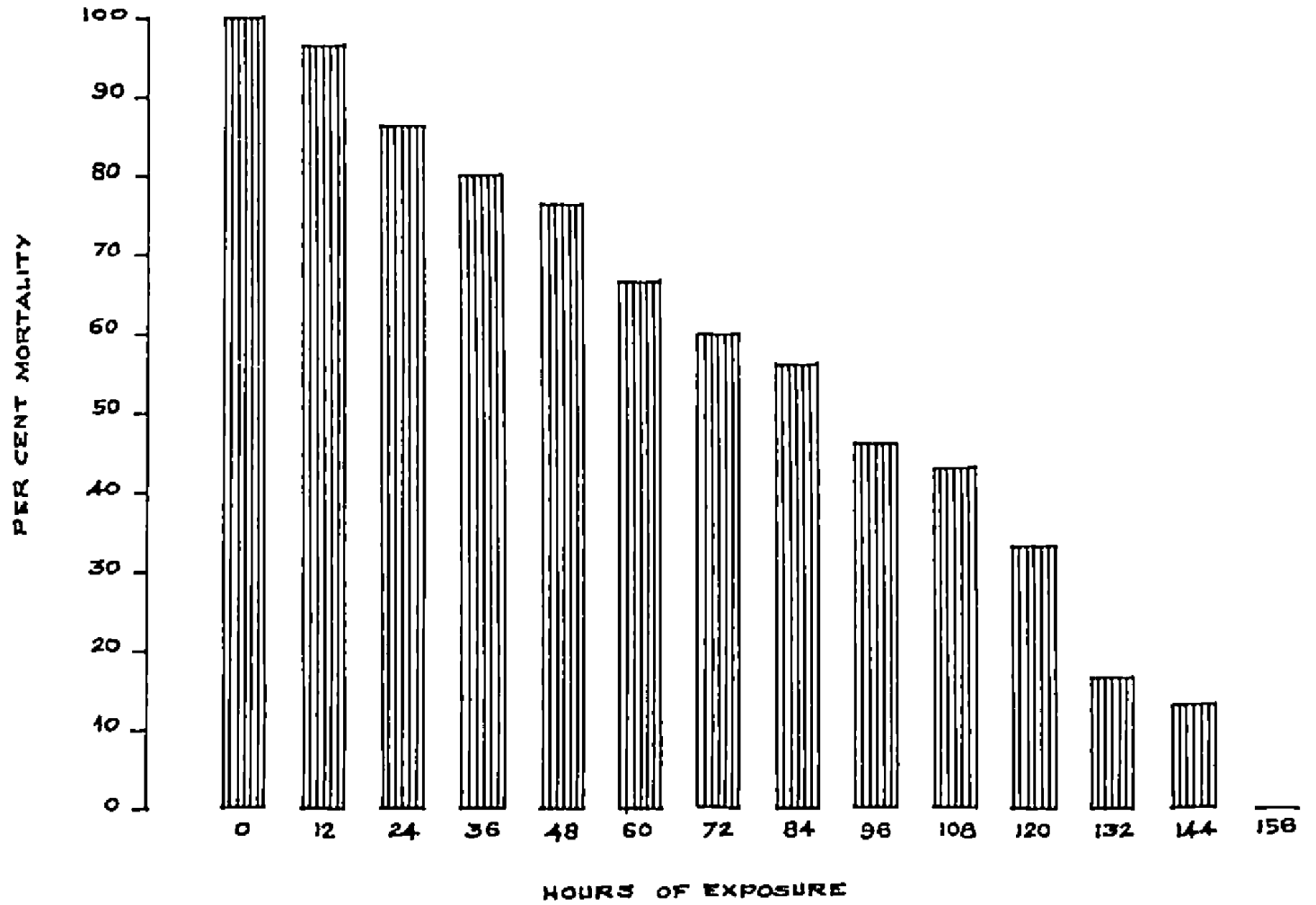

100
90
80
70
60
50
40
30
20
10
0
PER CENT MORTALITY
0
12
24
36
48
60
72
84
96
108
120
132
144
156
HOURS OF EXPOSURE

FIG 57

The infectivity was fully lost at 156 hour of exposure. The LT_{50} values ranged from 3.89 days with unexposed virus to 11.72 days with the virus exposed to heat treatment for 84 hours. LT_{50} values for the virus exposed to heat treatment for more than 84 hours could not be calculated as the mortality percentages were below 50. The half life of the virus (Table 12) continuously exposed to a temperature of 35°C was found to be 87.10 hours.

4.7.2. Effect of infra-red radiation on the viral activity

The results of bioassay of the virus, exposed to infra-red rays for periods ranging from zero to 156 hours, against third instar larvae of O.grenoselle are presented in Table 10, appendix 1 and Fig.58.

The larval mortality due to nuclear polyhedrosis virus was reduced from 100 per cent for the untreated virus to zero level at 156 hour of exposure. The data showed that the exposure of the virus to infra-red radiation for 84 hours reduced the larval mortality to 56.67 per cent. Exposure beyond 84 hours caused gradual reduction in the larval mortality reducing it to 6.67 per cent at 144 hour. There was an increase in the mean time taken for death from 4.57 days to 12.00 days with the increase in the

Table 10. Effect of exposure of ♂ of *O. arenosella* to infra red rays on the viral activity.

	Period exposure (in hours)													
	0	12	14	36	48	60	72	84	96	108	120	132	144	156
Percentage of larval mortality	100 (90)*	96.67 (79.53)	96.67 (79.53)	86.67 (68.61)	83.33 (65.88)	70.00 (56.79)	63.33 (52.71)	56.67 (48.85)	46.67 (43.11)	36 67 (37.29)	23.33 (28.86)	13.33 (21.39)	6.67 (15.00)	0.00
Time taken for death (range)	3-7	3-7	3-7	4-8	4-8	5-9	5-9	6-10	7-11	7-11	9-12	10-13	11-13	
Mean time for death (days)	4.57	4.69	4.76	6.12	6.68	6.90	7.95	9.00	9.71	10.18	11.14	12.00	12.00	..
LT_{50} (days	3.69	3.99	4 06	5.86	6.59	7.32	8.29	9.57	.	.	..	..	..	..
Fiducial limits	3.39 3.96	3.77 4.23	3.83 4 31	5.61 6 16	6.23 7.12	7.02 7.74	8.18 10.72	9.51 10.96	..	..	..	..	.	..
Heterogenity χ^2	3.34	4.78	1.99	1.23	1.89	1.79	2.15	1.10	..	.	..	..	.	..
Regression equation	A	B	C	D	E	F	G	H						

A = Y = 6.245x + 1.242 B = Y - 5.519x + 1.870 C = Y= 5.848x + 1.437 D = Y = 7.637x -0.863

E = Y = 5.476x + 0.513 F = Y = 5.534x - 1.518 G = Y= 5.702x - 0.582 H = Y = 8.057x -3.157

CD = 14.16

* Figures in the parenthesis are values after angular transformation

** The data were homogenous at P = 0.05

Fig. 58. Effect of exposing nuclear polyhedrosis virus of Opisina arenosella to infra red rays for different durations.

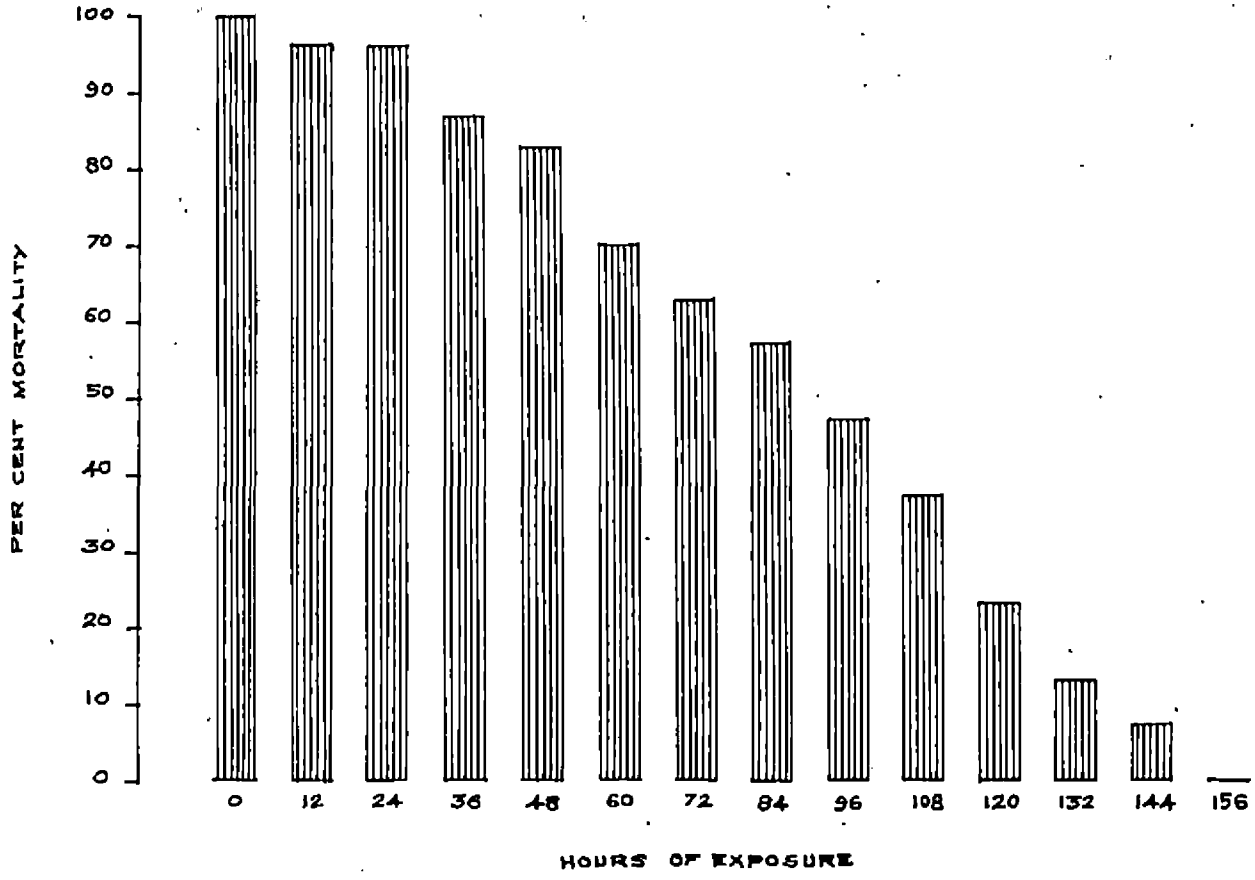

PER CENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
HOURS OF EXPOSURE

FIG. 58

duration of exposure upto 144 hours. The LT_{50} values ranged from 3.69 days for the unexposed virus to 9.57 days for the virus exposed to infra-red radiation for 84 hours. The LT_{50} values for the virus exposed to infra-red treatment for more than 96 hours could not be calculated since the per cent mortalities observed at these intervals were below 50. The half life of the virus when subjected to infra-red radiation was estimated to be 85.22 hours (Table 12).

4.7.3. Effect of ultra violet rays on the viral activity

Table 11, appendix 1 and Fig.59 presents the result of bioassay of the virus exposed to ultra violet rays for periods ranging from zero hour to 96 hours against third instar larvae of O. arenosella.

The infectivity of the virus was seen completely lost on exposure to ultra violet rays for 108 hours. The per cent mortality of larvae decreased from 100 to 6.67 per cent when the time of exposure of the virus to UV rays increased from zero to 96 hours. The exposure of the virus beyond 48 hours caused rapid reduction in the infectivity and larval mortality was brought down to 6.67 per cent at 96 hour of exposure. The mean time to death got prolonged from 4.53 days with unexposed virus to 11.50 days with

Table 11. Effect of exposure of NPV of O. arenosella to ultra violet rays.

	Period of exposure (In hours)									
	0	12	24	36	48	60	72	84	96	108
Percentage of larval mortality	100 (90)*	86.7 (68.61)	76.67 (61.64)	70.00 (56.79)	56.67 (48.85)	43.33 (41.15)	26.67 (31.11)	16.67 (24 12)	6.67 (15.00)	0 0
Time taken for death (range)	3-7	3-7	3-7	4-8	5-9	6-10	7-11	8-12	10-14	.
Mean time taken for death (days)	4.53	4.64	4.87	6.43	7.88	8.85	10,13	11.00	11.50	.
LT_{50} (days)	3.69	4.36	5.01	7.05	8.64	..	..	..	..	..
Fiducial limits	3 42 3.94	4.10 4.67	4.66 5 34	6.57 7 77	8.04 11.48	..		..	..	..
Heterogenity X^2	3.40	2.55	1.05	3.67	1.14	..	..	.	..	.
Regression equation	A	B	C	D	E					

A = Y = 5.088x + 1.833 B = Y = 5 142x + 1.425 C = Y = 5.012x + 0.006 CD = 12.94

D = Y = 5.140x + 1.712 E = Y = 5.107x + 0.592

* Figures in parenthesis are value after angular transformations

** The data were homogenous at P = 0.05

Fig. 59. Effect of exposing nuclear polyhedrosis virus of *Opisina arenosella* to Ultraviolet rays.

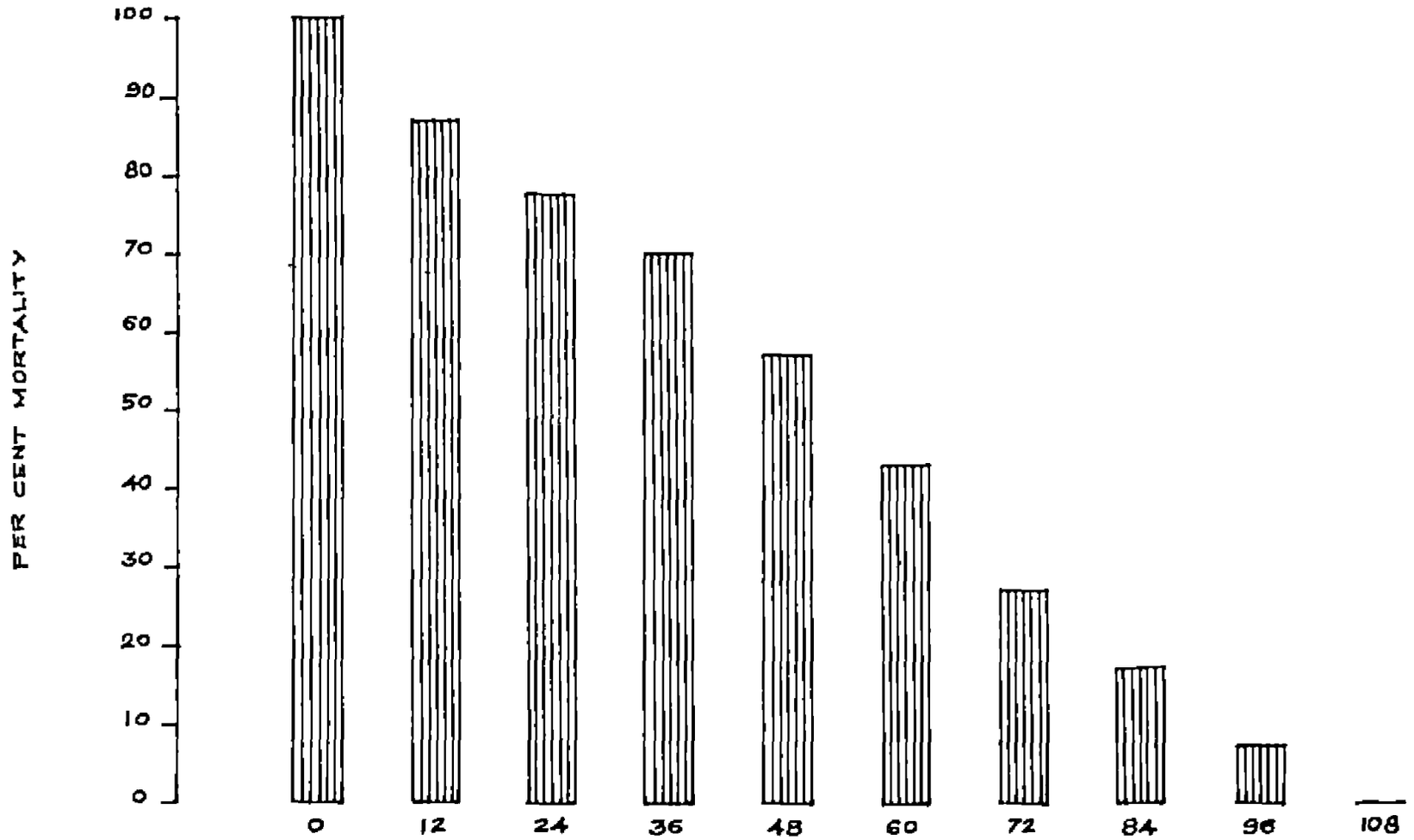

PER CENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
84
96
108
HOURS OF EXPOSURE

FIG 59

virus exposed to UV rays for 96 hours.

The LT_{50} values increased from 3.69 days with unexposed virus to 8.64 days for the virus exposed to UV treatment for 84 hours. The LT_{50} values for the virus exposed to UV radiation for more than 48 hours could not be calculated as the per cent mortalities at these intervals fell below 50. The half life of the virus exposed to UV rays was found to be 54.43 hours (Table 12).

4.8. Safety of the pathogen

4.8.1. Safety to silkworm, Bombyx mori

Table 13 presents the data on the effect of inoculating the nuclear polyhedrosis virus of G. areneosella to the third instar larvae of mulberry silkworm, Bombyx mori. All the third instar larvae tested pupated normally and recorded 100 per cent adult emergence. No mortality was observed in control also. The mean weights of the cocoons were 1.560 and 1.530 g for the nuclear polyhedrosis virus fed and for the control group respectively and the mean pupal periods were 14.71 and 14.92 days respectively. The mean number of eggs laid per female under treatment and check was 513 and 507 respectively.

Table 12. Half life of NPV of O. gronosella exposed to various physical factors of the environment.

Treatments	Hetero-genity	Regression equvation	Half life (hours)	Fiducial limits
Exposure to constant temperature (35°C)	4.690	Y = 2.21x + 3.83	87.10	73.79 99.54
Exposure to Infra red rays	5.870	Y = 2.54x + 4.14	85.22	75.61 93.35
Exposure to Ultra violet light	2.760	Y = 1.93x + 3.75	54.43	41.39 66.58
Weathering on coconut foliage	1.053	Y = 0.590x + 3.58	83.25	70.26 89.17

The data were homogenous at P = 0.05

Table 13. Effect of oral feeding of NPV of O. *arenosella* to Mulberry silkworm, *Bombyx mori*

Sl. No.	Characteristics	Treated	Untreated check
1.	Per cent mortality due to virus infection	0	0
2.	Percentage of larval survival	100	100
3.	Average cocoon weights (g)	1.560	1.530
4.	Mean pupal periods (days)	14.71	14.92
5.	Percent of adult emergence	100	100
6.	Mean fetundity	513	507

4.8.2. Safety to Bracon brevicornis

The data on the general behaviour of the parasitoid, per cent parasitisation of the larvae of O. arenosella, number of cocoons visible and per cent mortality of adult parasite are furnished in Table 14.

The parasitisation of the larvae of O. arenosella infected with nuclear polyhedrosis virus and those in control were 100 per cent respectively. The numbers of cocoons visible after the 9th day of liberation of the parasitoids were 40 and 41 in the treated and control groups respectively. The general behaviour of the parasitoids in the treatment and control was normal and no mortality was observed among them. There was no mortality among the progeny of B. brevicornis obtained from nuclear polyhedrosis virus infected larvae of O. arenosella and those from uninfected larvae.

When the adults of B. brevicornis fed with nuclear polyhedrosis virus, were released on the larvae of O. arenosella the number of cocoons visible after 9th day of liberation were 39 against 40 in control. No mortality of parasitoid was observed in treatment or in control and the general behaviour was normal. The larvae of O. arenosella parasitised was 100

per cent in the treatment and control groups.

4.8.3. Safety to Trichospilus pupivora

Table 14 presents the data on behaviour of the parasitoid, per cent mortality of adult parasitoid, number of adults emerged after 14th day of setting and per cent mortality in the progeny.

It was seen that the general behaviour of the parasitoid was normal and 100 per cent of the pupae exposed were parasitised in treatment and control. No death of the adult parasitoid due to viral infection was observed. The mean numbers of adult parasitoid emerged per pupa, 14 days after setting, were 520 and 525 in nuclear polyhedrosis virus infected and control lots respectively.

4.8.4. Safety to white rats and white mice

Data relating to the experiment are furnished in Table 15.

There was no difference in the appearance, behaviour and feeding activity of the test animals and control. No death occurred in any of the virus fed or water fed animals during the period of observation. Body temperature was found to be normal during the entire test period in both

Table 14. Effect of NPV of O. arenosella on Bracon brevicornis and Trichospilus pupivora

Observations	B.brevicornis exposed to NPV infected hosts*	B.brevicornis exposed to uninfected hosts*	Adults of NPV fed B.brevicornis exposed to uninfected hosts*	Normal adults of B.brevicornis exposed to uninfected hosts*	NPV fed T.pupivora adults exposed to healthy host pupae **	Normal T.pupivora adults exposed to healthy host pupae **
No.of larvae under test	10	10	10	10	50	50
No.of parasitoids liberated as seed	5 pairs	5 pairs	5 pairs	5 pairs	20	20
General behaviour of the parasitoids	Normal	Normal	Normal	Normal	Normal	Normal
Percent parasitisation of the host	100	100	100	100	100	100
Per cent mortality of adult parasitoids	Nil	Nil	Nil	Nil	Nil	Nil
No.of cocoons visible/adult emerged	40	41	39	40	520	525
Mortality among progeny	Nil	Nil	Nil	Nil	Nil	Nil

* Means of 5 replications

** Mean of 3 replication

Table 15. Effect of feeding white rats and white mice with the nuclear polyhedrosis virus of O.arenosella.

Observations	White rats		White mice	
	Treated	Control	Treated	Control
Initial mean weight of the animal (g)	107.400	109.000	25.000	26.980
Final mean weight of the animal (g)	111.400	112.500	27.200	29.410
Mean weight gain (g)	4.000	3.500	2.200	2.430
Body mean temperature	38.4°C	38.3°C	37.4°C	37.6°C
Haemoglobin (g/100 ml)	13.60	14.20	10.60	10.40
Lymphocytes	62.25	64.50	47.40	50.20
Neutrophils	35.25	32.00	49.60	48.30
Eosinophils	2.50	3.50	7.80	1.90
Basophils	-	-	1.40	1.30
Monocytes	-	-	1.30	1.50
Total count	6900/c mm	6800/c mm	2500/c mm	2600/c mm
Erythrocyte sedimentation rate (ESR)	0 mm/hr	0 mm/hr	0 mm/hr	0 mm/hr
Organ wet weight (mg) Heart	0.499	0.405	0.126	0.121
Lungs	1.753	1.789	0.166	0.148
Liver	3.543	3.473	1.155	1.110
Kidney	0.963	0.973	0.411	0.432
Spleen	0.507	0.520	0.118	0.108
Testes	0.072	0.075	0.111	0.118
Ovary	-	-	0.066	0.067

* Mean of 5 replications

the groups. Body weight gained in virus treated white rats and white mice also was on par with those of untreated animals. No difference due to virus treatment could be detected in total or differential counts of blood cells, or in erythrocyte sedimentation rate. Post-mortem examination of various organs did not reveal any gross pathological conditions caused by the treatment. The average weights of the organs also did not vary. Histological examination of the organs did not show any evidence of tissue damage.

4.8.5. Infectivity to the developing chick embryo

The results showed that the nuclear polyhedrosis virus did not cause any mortality of the developing embryo. No death occurred in any of the virus treated or normal saline treated chick embryo during the entire test period. All the developing embryos were found healthy and there was no change in their appearance and behaviour. At the end of the test period the embryos were killed by keeping them in a deep freezer and post-mortem examination did not show any gross pathology in the internal organs. Histological examinations of the tissues also showed no evidence of damage.

4.9. Evaluation of the efficacy of the virus in controlling O.arenosella incidence in field

4.9.1. Preliminary field experiment

The data relating to the experiment are furnished in Table 16. The higher larval mortality of 85.00 per cent was recorded in plot treated with Thiodan 0.05 per cent spray and it was closely followed by nuclear polyhedrosis virus sprayed at 22.14 x 10^7PIBs/ml with larval mortality of 75.00 per cent, there being no statistically significant difference between the two. The mortality in plots treated with nuclear polyhedrosis virus at 22.14 x 10^6PIBs/ml was 66.25 per cent and it was on par with the mortality in the higher concentration of 22.14 x 10^7PIBs/ml. Mortality in control plot was 3.75 per cent only.

4.9.2. Main field experiment

The data relating to the experiment are presented in Table 17 a to c, appendix III and illustrated in Fig.60.

The data relating to the experiment in location I are presented in Table 17 a and illustrated in Fig. 60 a. The nuclear polyhedrosis virus as well as Thiodan gave significant reduction in larval population during the first week after spraying. The population in plots treated with thiodan and nuclear polyhedrosis virus were 23.64 and 29.46

Table 16. Percentage mortality of third instar larvae of O. arenosella with NPV and Thiodan in field

Treatments	Mean percentage mortality of larvae		Mean percentage mortality of pupae	Mean percentage of adults emerged
22.14 x 10^7 PIBs/ml	75.00	(60.33)*	Nil	24.50
22.14 x 10^6 PIBs/ml	66.25	(54.51)	Nil	33.75
22.14 x 10^5 PIBs/ml	48.75	(44.31)	Nil	51.25
22.14 x 10^4 PIBs/ml	26.25	(30.85)	Nil	73.75
Thiodan 0.05 per cent	85.00	(67.21)	Nil	15.00
Control	3.75	(11.24)	Nil	96.25

CD - 7.75

* Figures in parenthesis are transformed values in angles

per tree respectively while in control the population was as high as 62.77 larvae per tree. Thereafter a gradual increase in mean larval population was observed in treated plots upto the seventh week, while in control the population remained at the initial level. At seventh week the mean larval counts were 62.24, 46.83 and 62.30 in Thiodan treated, nuclear polyhedrosis virus treated and control plots respectively. In subsequent observations the population declined in all treatments including control. The population build up in nuclear polyhedrosis virus treated plots was much lower than that in Thiodan treated plot. On the sixth week itself the population in Thiodan treated plots (62.32) came on par with that of control (62.65) while the population in nuclear polyhedrosis virus treated plot was significantly lower (43.76). Even at the last observation on the fourteenth week the population in nuclear polyhedrosis virus treated plot was significantly lower than that of Thiodan treated plots and control, the population in the three plots being 35.98, 42.90 and 44.77 respectively.

The data relating to the experiment in location II are presented in Table 17b and illustrated in Fig. 60b. As in location I substantial reduction in population was observed in the virus and Thiodan treated plots during

Table 17 Population of O. araneosella in field treated with NPV, Thiodan and water (control)

(a) Location I

Treatments	Mean no of larvae per tree before treatments	Mean number of larvae per tree observed at different intervals after spraying (in weeks)														Mean
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Thiodan 0.05%	63.11 (7.98)*	23.64 (4.86)	25.96 (5.10)	36.68 (6.06)	47.84 (6.92)	57.62 (7.59)	62.32 (7.89)	62.24 (7.89)	53.52 (7.32)	50.57 (7.11)	49.86 (7.06)	47.03 (6.86)	43.84 (6.62)	42.15 (6.49)	42.90 (6.55)	46.57 (6.82)
NPV 22.14×10^7 PIBs/ml	63.09 (7.94)	29.48 (5.43)	31.42 (5.61)	32.24 (5.68)	33.91 (5.82)	39.16 (6.20)	43.76 (6.62)	46.93 (6.84)	35.12 (5.93)	32.36 (5.69)	32.29 (5.68)	39.59 (6.97)	37.97 (6.16)	35.88 (5.99)	35.90 (6.00)	37.58 (6.13)
Control	62.57 (7.91)	62.77 (7.92)	62.86 (7.93)	61.12 (7.82)	60.45 (7.77)	61.14 (7.82)	62.65 (7.88)	62.30 (7.89)	56.01 (7.48)	52.23 (7.23)	51.75 (7.19)	49.55 (7.04)	46.77 (6.84)	44.62 (6.68)	44.77 (6.69)	55.80 (5.47)
C D		0.07	0.09	0.06	0.09	0.07	0.08	0.05	0.09	0.11	0.11	0.12	0.15	0.13	0.15	0.13

C D for comparison of treatments at various periods = 0.09

(b) Location II

Thiodan 0.05%	63.66 (7.98)	23.34 (4.83)	27.76 (5.27)	37.04 (6.09)	44.05 (6.64)	53.92 (7.34)	59.20 (7.69)	60.86 (7.80)	54.07 (7.35)	46.69 (6.83)	49.11 (7.01)	46.76 (6.69)	42.99 (6.56)	42.75 (6.54)	42.28 (6.50)	45.70 (6.76)
NPV 22.14×10^7 PIBs/ml	62.85 (7.92)	24.76 (4.94)	27.60 (5.25)	33.09 (5.75)	36.26 (6.02)	40.06 (6.34)	41.89 (6.41)	46.47 (6.82)	35.44 (5.95)	33.18 (5.76)	32.43 (5.69)	32.02 (5.66)	32.32 (5.66)	32.05 (5.66)	32.00 (5.66)	35.64 (5.97)
Control	62.96 (7.93)	62.87 (7.93)	63.09 (7.94)	62.79 (7.92)	63.15 (7.95)	63.47 (7.97)	63.46 (7.97)	63.62 (7.98)	55.56 (7.45)	51.34 (7.17)	50.71 (7.12)	49.52 (7.04)	48.51 (6.96)	46.69 (6.83)	46.16 (6.79)	56.70 (7.53)
C D		0.08	0.12	0.13	0.08	0.07	0.07	0.09	0.11	0.11	0.11	0.1	0.16	0.15	0.12	0.38

C D for comparison of treatments at various periods = 0.10

(c) Location III

Thiodan 0.05%	60.59 (7.78)	22.36 (4.72)	25.50 (5.05)	33.78 (5.85)	33.74 (5.81)	39.01 (6.25)	42.76 (6.54)	45.48 (6.74)	39.60 (6.29)	34.25 (5.85)	33.89 (5.65)	30.95 (5.56)	30.34 (5.51)	32.51 (5.70)	32.75 (5.71)	34.93 (5.91)
NPV 22.14×10^7 PIBs/ml	60.51 (7.78)	25.94 (5.09)	27.34 (5.23)	30.22 (5.50)	31.14 (5.58)	33.71 (5.81)	36.75 (6.06)	39.85 (6.31)	32.51 (5.70)	31.60 (5.62)	31.24 (5.59)	30.70 (5.54)	30.86 (5.56)	30.74 (5.54)	31.20 (5.59)	33.29 (5.77)
Control	60.20 (7.76)	59.81 (7.73)	61.14 (7.82)	61.01 (7.81)	61.26 (7.83)	61.40 (7.84)	61.15 (7.82)	60.87 (7.80)	52.23 (7.23)	50.82 (7.13)	50.35 (7.10)	50.25 (7.09)	48.11 (6.94)	46.65 (6.84)	46.05 (6.79)	55.20 (7.43)
C D		0.10	0.09	0.05	0.07	0.08	0.08	0.07	0.10	0.09	0.08	0.07	0.11	0.15	0.14	0.30

C D for comparison of treatments at various periods = 0.09

* Figures in the parenthesis were values after $\sqrt{x}$ transformation

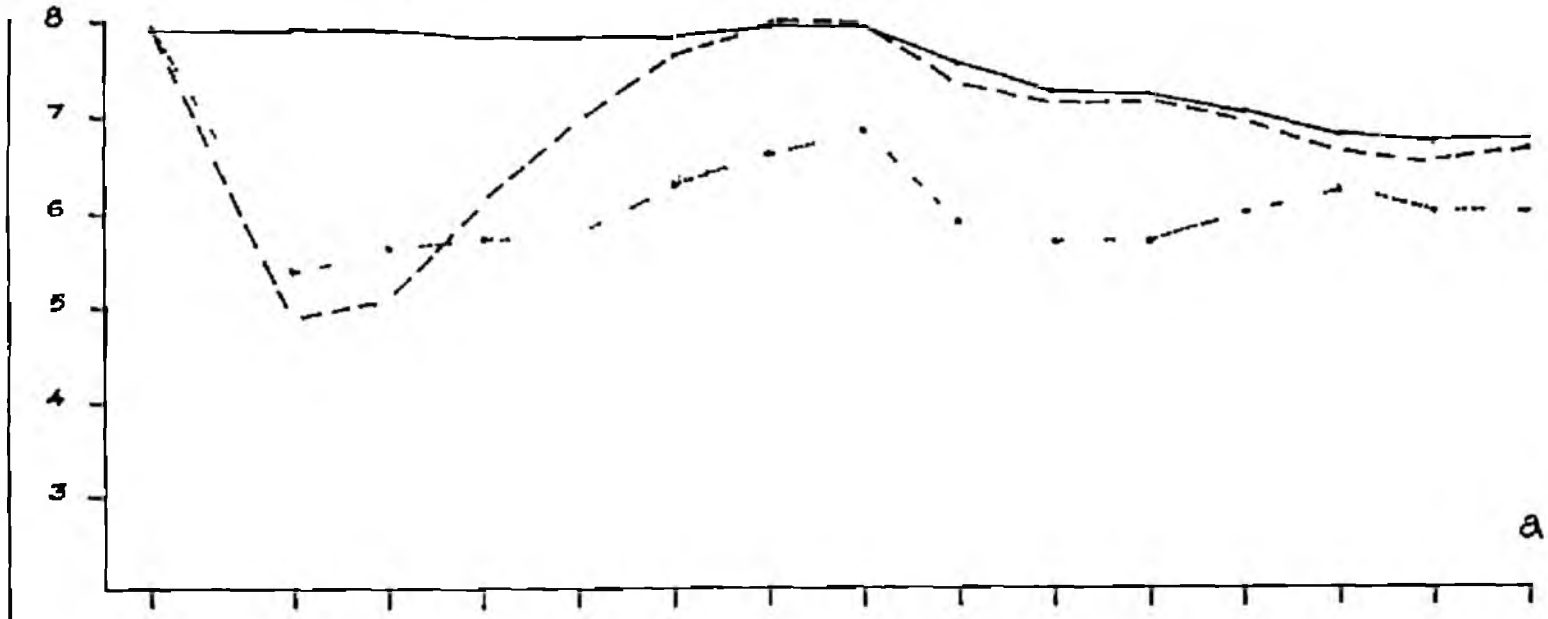
Fig. 60. Effect of spraying nuclear polyhedrosis virus and thiodan on the population of Opisina arenosella in field.

a. Location I

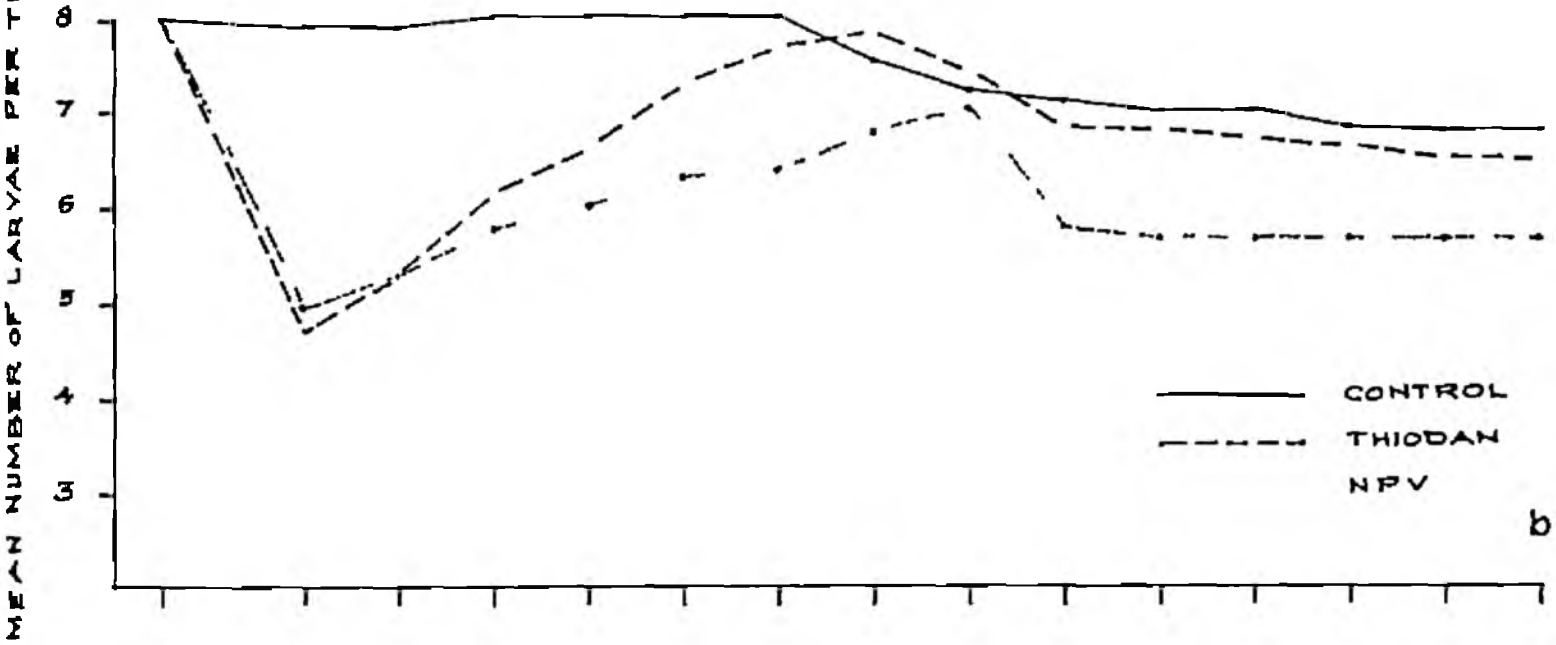
b. Location II

c. Location III

LOCATION - I

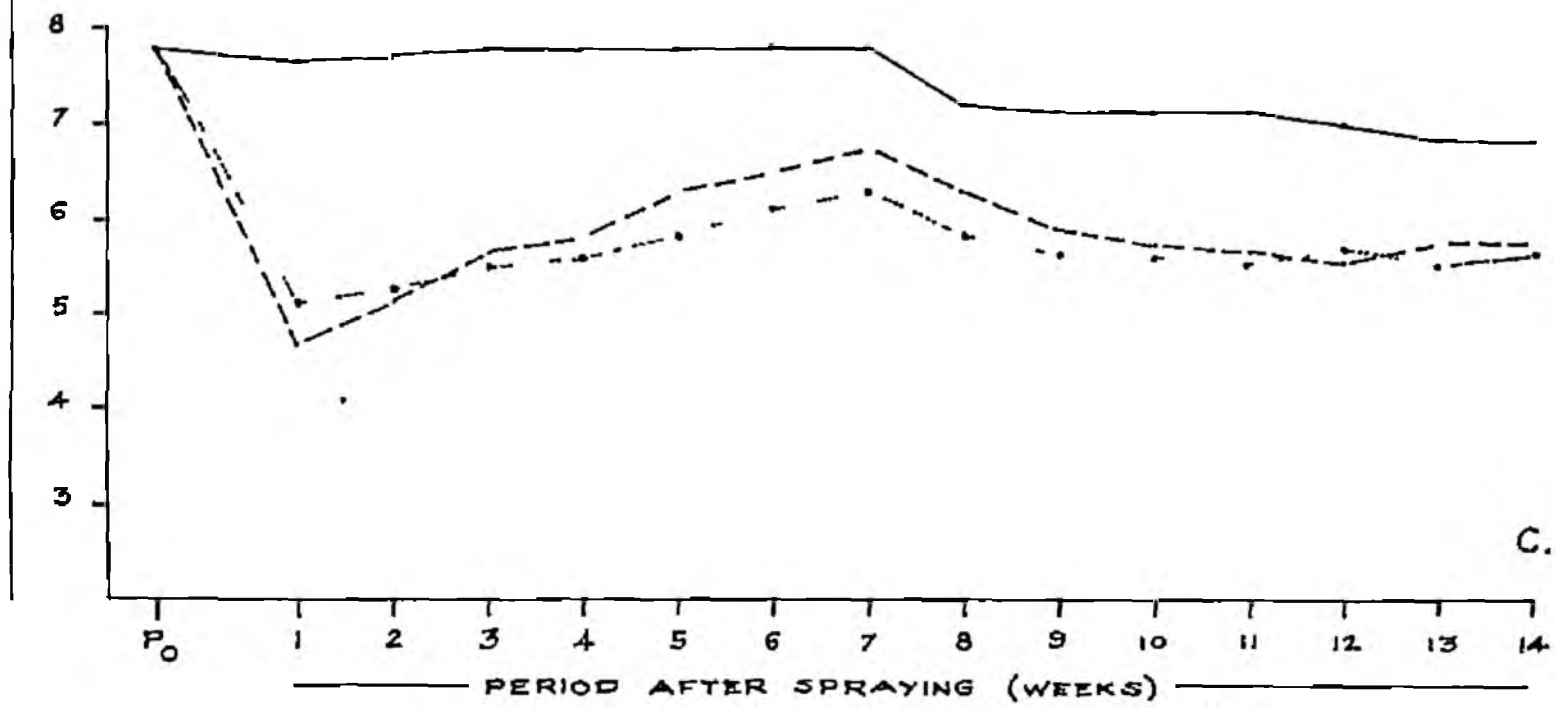


LOCATION II



MEAN NUMBER OF LARVAE PER TREE

LOCATION - III



PERIOD AFTER SPRAYING (WEEKS)

P_0 — LARVAL COUNT PRIOR TO SPRAYING

FIG 60

the first week after treatment. The population in plots treated with Thiodan and nuclear polyhedrosis virus were 23.34 and 24.36 per tree respectively while in control the population was as high as 62.87 per tree. A gradual increase in pest population was observed upto the seventh week in treated plots while in control the population remained at the initial level. At the seventh week the mean larval counts were 60.86, 46.47 and 63.62 in thiodan treated, nuclear polyhedrosis virus treated and control plots respectively. In subsequent observations the population declined in all treatments including control. The population build up in nuclear polyhedrosis virus treated plot was much slower than that in Thiodan treated plots. On the eighth week the population of Thiodan treated plots (54.07) came on par with that of control (55.56) while the population in nuclear polyhedrosis virus treated plots remained significantly lower (35.44). During the fourteenth week after treatment also the population in nuclear polyhedrosis virus treated plot was significantly lower than that of Thiodan treated plots and control, the mean population in three plots being 42.28, 32.00 and 46.16 per tree respectively.

The data relating to the experiment in location III are presented in Table 17 c and illustrated in Fig.60 c. Nuclear polyhedrosis virus as well as thiodan gave significant reduction in larval population during the first week after spraying. The population in plots treated with thiodan and nuclear polyhedrosis virus were 22.30 and 25.94 per tree respectively while in control the population was as high as 59.81. A gradual increase in pest population was observed in treated plots in subsequent weeks. At the seventh week the mean larval counts were 45.48, 39.85 and 60.87 in Thiodan treated, nuclear polyhedrosis virus treated and control plots respectively. In subsequent observations the populations declined in all treatments including control. The population build up in nuclear polyhedrosis virus treated plots was much slower than the build up in thiodan treated plots. In the tenth week the population in Thiodan treated plots (31.89) came on par with that of nuclear polyhedrosis virus treated plot (31.24) while the population in control plots was significantly higher (50.35). During the fourteenth week after treatment the population in control was significantly higher than that of Thiodan and nuclear polyhedrosis virus treated plots, the population in the three plots being 46.05, 32.75 and 31.20 respectively.

4.9.3. Incidence of parasitoids in nuclear polyhedrosis virus and Thiodan treated plots and in control

Data on the extent of natural parasitism of O. aronosella larva in plots treated with nuclear polyhedrosis virus and Thiodan are furnished in Table 18a and b, appendix IV and illustrated in Fig. 61a and b. It was seen that there was 40.18 per cent parasitism in the insecticide treated plots and 39.20 per cent parasitism in the nuclear polyhedrosis virus treated plots while the extent of parasitism in the untreated control plot was 38.80 per cent. There was no significant difference among the treatments with reference to the extent of parasitism. The per cent parasitisation by Trichospilus pupivora in plots treated with Thiodan, nuclear polyhedrosis virus and water (control) were 3.16, 3.56 and 2.84 respectively while that of Brachymeria spp. were 37.02, 35.72 and 35.92 respectively.

The intensity of natural parasitism estimated from observation of empty pupal cases collected from treated and untreated plots are presented in Table 18b and illustrated in Fig.61b. It was seen that there was 44.30 per cent parasitism in the insecticide treated plots and 44.60 per cent parasitism in the nuclear polyhedrosis virus

Table 18a. Effect of spraying NPV and thiodan on natural parasitisation of the pupae of O. grenosella collected from field and reared in laboratory.

Treatments		Percentage parasitisation observed at different intervals after spraying (fortnight)					
		1	2	3	4	5	Mean
Thiodan	a)	4.20	3.10	2.20	2.70	3.60	
	b)	40.20	31.30	30.00	40.60	43.00	
	c)	44.40 (41.80)*	34.40 (35.90)	32.20 (34.58)	43.30 (41.16)	46.60 (43.08)	40.18 (39.31)
NPV	a)	2.70	4.70	1.90	3.80	4.70	
	b)	40.60	29.70	30.20	44.00	34.10	
	c)	43.30 (41.16)	34.40 (35.90)	32.10 (34.54)	47.80 (43.72)	38.80 (38.57)	39.20 (38.78)
Control	a)	2.70	3.80	2.20	2.60	2.90	
	b)	37.30	34.90	30.90	41.70	34.80	
	c)	40.00 (39.22)	38.70 (38.51)	33.10 (35.13)	44.30 (41.79)	37.70 (37.91)	38.80 (38.52)

a: Trichospilus pupivora b: Brachymeria spp: c: Total

* Figures in the parenthesis are transformed values in angles

Table 18b. Intensity of natural parasitism of O. arenosella pupae as observed on the basis of the emergence holes in the empty pupal cases collected from field

Treatments		Per cent parasitisation of pupae at fortnightly intervals					
		1	2	3	4	5	Mean
Thiodan	a)	3.80	3.70	2.90	2.30	3.10	
	b)	50.70	39.60	30.40	49.90	32.00	
	c)	54.50 (47.56)*	43.30 (41.16)	33.30 (35.28)	52.20 (46.29)	35.10 (36.35)	44.30 (41.74)
NPV	a)	4.90	3.80	2.70	3.70	4.70	
	b)	52.90	47.30	28.30	40.80	40.80	
	c)	57.80 (49.49)	51.10 (45.64)	31.00 (33.86)	44.50 (41.81)	45.50 (42.42)	44.60 (41.88)
Control	a)	2.90	3.30	2.10	3.90	3.70	
	b)	46.00	43.30	27.80	41.60	39.50	
	c)	48.90 (44.36)	46.60 (43.07)	29.90 (33.19)	45.50 (42.42)	43.20 (41.11)	43.40 (41.22)

a: Trichospilus pupivora b: Brachymeria spp: c: Total

* Figures in the parenthesis are transformed values in angles

Fig. 61. Parasitisation observed in nuclear polyhedrosis virus and Thiodan treated coconut plantations.

a) Assessed from the live pupae collected from field.

b) Assessed from the empty pupal cases collected from field.

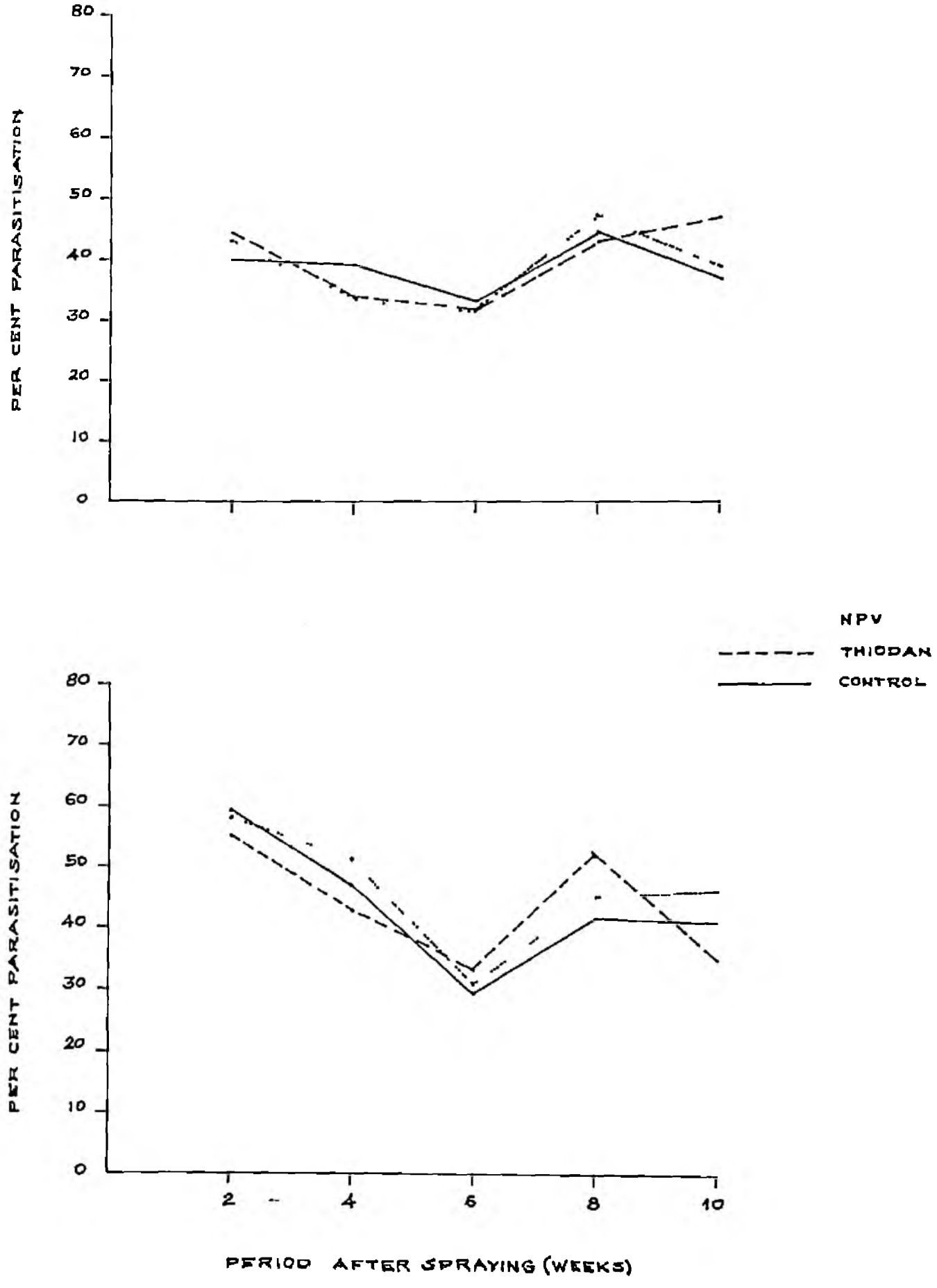

80
70
60
50
40
30
20
10
0
PER CENT PARASITISATION
NPV
THIODAN
CONTROL
2
4
6
8
10
PERIOD AFTER SPRAYING (WEEKS)

FIG 61

treated plot while the extent of parasitisation in the untreated control plot was 43.40 per cent. There was no significant difference in the extent of parasitism. The per cent parasitism by Trichospilus pupivora in plots treated with Thiodan, nuclear polyhedrosis virus and water (control) were 3.16, 3.96 and 3.18 respectively while that of Brachymeria spp. were 40.52, 42.02 and 39.64 respectively.

DISCUSSION

DISCUSSION

A nuclear polyhedrosis virus of Opisina arenosella was first recorded in India by Philip et al., (1982). Basic informations required for developing a pest control strategy using the pathogen were lacking. The present investigations were carried out with the objective of collecting these informations.

The symptomatology of the disease, nature of the virus, and its mode of transmission, cross-infectivity to other species of lepidoptera, bioassay of viral activity, effect of environmental factors on the viral activity, the safety of the virus to non-target organisms and its efficacy in controlling the pest in field were investigated.

The symptomatology was studied in detail with a view to facilitating the identification of infected larval stages in field populations of the pest. The symptoms of the disease observed resembled the general symptoms of nuclear polyhedrosis of lepidopterous larvae detailed in the reviews done by Aizawa (1963) and Smith (1967). The infected larvae became paler than normal healthy ones and soon they became lethargic and showed

a loss of appetite. They became bloated in three to four days after infection. In some cases, a dark brown fluid was seen discharged through the mouth. In later stages of infection, the body of the larvae became oily and they stopped their feeding and movement. The whole body content became liquefied and milky white in colour. The colour could be seen through the cuticle. The cuticle became fragile and ruptured even with slight pressure liberating the liquefied body content containing the polyhedra in large numbers. Usually the lepidopteran larvae infected by nuclear polyhedrosis virus came over with the head hung downwards at the time of death and remained in the open. This behaviour was not observed in the case of O. arenosella. Such exceptions were seen earlier in Heliothis peltigera (Harpaz and Zlotkin, 1965) and in H. armigera (Jacob, 1972) also.

When the infection of O. arenosella occurred in the late larval instars, the mortality occurred in the pre-pupal/pupal stage. Similar observations were made by Hafez (1958) in Prodenia litura and Tanada (1954) in Pieris rapae. Vail and Hall (1969b), Vail and Gough (1970) in cabbage looper, Trichoplusia ni and Mathew (1980) in Anadevidia peponis infected with nuclear polyhedrosis viruses.

Larvae infected with sublethal doses of the virus pupated and moths emerged from them. But many of these moths were malformed and their tissues contained polyhedral inclusion bodies. Theemergence of such malformed adults was observed by Hafez (1958) in P. litura, Vail and Hall (1969[b]) in cabbage looper, T. ni and Mathew (1980) in A. peponis.

Electron microscopic observations showed that the diameter of the polyhedra of O.arenosella ranged from 533.33 nm to 1666.67 nm with an average of 1393.30 nm. Smith (1967) had concluded that the diameter of the polyhedra generally ranged from 500 nm to 1500 nm depending upon the host species. But the diameter of the inclusion bodies of P. litura ranged from 2500 nm to 4000 nm (Ramakrishnan and Tiwari, 1969) and of the polyhedra of Buzura suppressaria ranged from 600 to 2600 nm (Chan et al., 1981). The size of the polyhedra of O.arenosella was thus found to be within the normal limits. Varying number of virus rods occurred in bundles in each developmental membrane within the polyhedra and hence the pathogen was identified as a multiple embedded virus.

The tissues of the larvae of O.arenosella infected by the virus were hypodermis, fat body, trachea, muscles,

gut epithelium, connective tissues surrounding the gut, Malpighian tubules, nerve ganglia and epithelial sheath of gonads. The hypodermis, fat body and tracheal matrix cells were recognized as the principal sites of multiplication of nuclear polyhedrosis virus (Aizawa, 1963; Smith, 1967). In O. arenosella also the above tissues were found to be the major sites of infection. In addition to these major sites, virus multiplication was observed in a number of other tissues mentioned above. Infection of these tissues was reported previously also in some Lepidoptera. Tanada (1959) observed infected muscle cells of Pseudaletia unipuncta. Benz (1963) reported infection of nerve sheath, ganglion cells and muscle cells of Malacosoma alpicola while Adams et al., (1968) observed polyhedra in the nerve cord and connective tissues surrounding the ganglion of Ceramica picta. Hamm (1968) found infection of epithelial lining of foregut and ganglia of S. frugiperda. Mathad et al., (1968) found infection of neurilemma of nerves, ganglia, sarcolemma connective tissues surrounding the mid gut and wing buds in P. ni.

Presence of polyhedra observed in the midgut epithelium in O. arenosella is a phenomenon a rare in

lepidopteran larvae infected by nuclear polyhedrosis virus. Midgut epithelium was seen infected in T. ni (Heimpel and Adams 1966) and in Mythimna separata (Sugun et al., 1983) exposed to nuclear polyhedrosis viruses.

No infection in the tissues of silk gland, brain, pericardial cells and wing buds could be detected in the present investigations.

The nature of infection in the hypodermis of O. arenosella did not differ from the previously reported cases. But the highly conspicuous thickening of the hypodermis observed in the present study is not a common feature. The normal hypodermal tissues measured 1320 nm in thickness (mean) with a range of 1220 nm to 1470 nm, while the infected hypodermis had a mean thickness of 2450 nm with a range of 1980 nm to 3020 nm. However Watanabe (1968) had reported an abnormal proliferation of cells in the epidermis of the fall web worm, Hyphantria cunea induced by infection of a nuclear polyhedrosis virus.

Another striking feature observed in the histopathological studies was the asynchronous infection of the fat body cells. In later stages of infection even the

adjacent cells in a region of the fat body were in entirely different stages of infection. All stages ranging from those of stroma and ring zone formation to the rupture and release of polyhedral bodies from the nuclei could be seen. Lack of uniformity in the infection of fat body cells was reported by Harpaz and Zlotkin (1965) in H. peltigera, Hunter and Hall (1968) in S. exigua, Jacob and Subramaniam (1972) in P.litura and Sugun et al., (1983) in Mythimna separata. They observed that the phenomenon might be due to the infective agent invading susceptible tissues differently at random or the cells of the tissue having inherent properties which caused the infection process to progress at different rates. A progressive reduction of fat vacuoles and their total disappearance in many areas of the fat bodies in the final stage were observed and these indicated a progressive utilization of stored fat by the infected larvae. The retarded feeding rate and lower utilization of food materials by infected larvae might have caused the depletion of stored fat.

The infection in all the tissues had reached the peak at 120 hour after inoculation. Then the disintegration and rupturing of cells which resulted in the release of polyhedra into the haemocoel were visible.

Infection of nerve ganglia and nerve cords observed could have caused the impairment of locomotion and lack of response to tactile stimuli and disruption of various other physiological functions observed in the larvae during the stage.

The histopathological studies thus showed the nuclear polyhedrosis virus of O. arenosella as a poly organotrophic virus. The extensive invasion of the larval tissues by the pathogen indicated its high virulence in the host.

The transmission of the virus through eggs of the host is of vital importance in the prospect of controlling an insect pest using the microbial organism. It is a vital means of dispersal which may cause repeated epizootics in a pest population and thus provide an economical and self-perpetuating mechanism of control. Ignoffo (1968) had rightly recognised its usefulness and recommended auto-dissemination, which involves the use of host insect to introduce and spread an entomopathogen in the ecosystem, as a successful method for the dispersal of a pathogen in the field.

A significant percentage of the caterpillars emerging from the eggs of moths, obtained from virus treated larvae

of O. arenosella, succumbed to viral infection and died when the eggs were not surface sterilized and the mortality became negligible when they were surface sterilized. The eggs laid by infected moths obviously had the virus on the surface of the chorion which might have gone into the emerging larvae when they bite open the egg shell at hatching. Mortality observed among the larvae hatching out of surface sterilized eggs might have occurred due to the incomplete sterilization since the percentage observed was very low and negligible. Doane (1969) described a similar transmission of the nuclear polyhedrosis virus of gypsy moth. When the homogenate of the eggs laid by the above infected moths taken without surface sterilization, was fed to the third instar larvae of O. arenosella, high mortality occurred while homogenate of surface sterilized eggs caused very low mortality. These results also indicated the transmission of the virus through surface contamination of the eggs.

The apparently normal moths emerging from a virus treated population of O. arenosella larvae laid a high percentage of infected eggs thus served to introduce and perpetuate the pathogen in succeeding generations. An apparent partial control achieved by the NPV of

O. arenosella might hence prove to be a complete one in ultimate effect.

A portion of the eggs laid by virus fed moths developed into larvae which succumbed to viral infection when the eggs were not surface sterilized and the mortality became negligible when they were surface sterilized. Vail and Hall (1969b) obtained virus transmission to the progeny of virus fed moths of cabbage looper and they also found that the infection could be eliminated by surface sterilization of eggs. Hamm and Young (1974) demonstrated the presence of polyhedra on the surface of eggs laid by virus fed Hyphantria cunea moths, using a scanning electron microscope.

When the genitalia of mated female moths were contaminated with the virus, the mortality of larvae emerging from eggs laid by them reached 41.89 per cent. In this case also the mortality could be completely eliminated by the surface sterilization of eggs. Similar observations were reported in the case of Colias eurytheme, T. ni (Martignoni and Hilstead, 1962), H. cunea (Elmore and Rowland, 1964) and in S. mauritia (Nair and Jacob, 1985). The possibility of naturally contaminated adults serving as virus carriers and helping in the dissemination of the virus in the field population is indicated by this result.

Thus, the above experiments showed that the moths which emerged from larvae exposed to sublethal doses of NPV and those contaminated internally through food or externally at the genitalia served as effective carriers of the pathogen and helped in the natural perpetuation and spread of the disease.

The feasibility of transovum transmission was indicated in the histopathological observations too. Viral infection was seen in the epithelial sheath of the gonads. The cells inside the follicles were not seen affected. The polyhedra present in the ovarian sheath might have contaminated the surface of ova when released from the follicles and this might have been the mechanism of transovum transmission of the pathogen observed in the experiments.

The cross-transmission studies showed that the nuclear polyhedrosis virus of O. arenosella were infective to the larvae of S. litura (Noctuidae), S. mauritia (Noctuidae), Anadevidia peponis (Noctuidae), Pericallia ricini (Arctiidae) and Diacrisia obliqua (Arctiidae) while the larvae of Orthaga exvinaceae, Euproctis fraterna, Sylepta derogata, Margaronia indica and Chaphalocrocis medinalis were not susceptible to the pathogen. The NPV of O. arenosella was transmissible to insects belonging to different families

of lepidoptera viz. noctuidae and arctidae. Similar interfamily and interspecific transmissions were noticed earlier also. Aizawa (1962) obtained successful cross transmission of the nuclear polyhedrosis virus of Bombyx mori (Bombycidae) to Galleria mellonella (Galleridae). Fraser and Stairs (1982) reported the cross transmission of the NPV of G. mellonella (Galleridae) to T. ni (Noctuidae), H. zea (Noctuidae) and Manduca sexta (Sphingidae).

Based on the recovery of PIBs/ml of body fluid the susceptible species of insect could be ranked in the following descending order of preference for mass multiplication of the virus, S. litura, S. mauritia, A. peponis, P. ricini and P. oblinua. S. litura and S. mauritia were sturdy and more amenable to laboratory rearing with shorter life cycles. They were hence preferred for large scale multiplication of the NPV in the laboratory. The virus thus multiplied in the alternate hosts repeatedly did not show any loss of infectivity or its virulence to O.arenosella. The alternate hosts themselves being major pests of economically important crops the possibility of using the virus as a broad spectrum microbial insecticide for plant protection also is indicated.

The stage susceptibility of an insect host to a pathogen has to be ascertained for the proper timing of introducing the pathogen in an ecosystem for bio-control and it is well known that the success of a microbial insecticide used in plant protection will depend largely on this strategy. It was hence felt essential to find out the optimum dose of the pathogen and the more susceptible stages of the pest for evolving a proper technology to control the pest in field. This was done by the bioassay of viral activity using the various larval instars as test organisms. The LC_{50} values increased with increase in larval age indicating that the susceptibility of the pest decreased with the increase in the age of the larvae. The LC_{50} for the fourth and fifth instars were 242.49 times and 3652.58 times that of the first instar larvae while those of the second and third instars were 24.38 and 113.01 times higher only. The results showed that first, second and third instars were susceptible to moderate doses of the nuclear polyhedrosis virus while the fourth and fifth instars required very hign doses of the virus for effective kill. Earlier studies also had shown similar results with other insect species (Clark and Thompson, 1954;

Abul-Nasr, 1959; Tanada and Reiner, 1962; Stairs, 1965b; Ignoffo, 1966a; Hunter and Hall, 1968; Lathika and Jacob, 1974a; Pawar and Ramarksihnan, 1975; and Nair, 1981). The mean time taken for death also was seen prolonged with increase in age of the larvae.

The observations on LC_{50} and LT_{50} values indicated that O. arenosella had to be exposed to large doses of the pathogen as they advanced in age. Such resistance associated with the growth of the larvae was regarded as maturation immunity by Tanada (1956). But Ignoffo (1966b) suggested that it might possibly be attributed to the normal increase in body weight which 'dilutes' a constant viral dose. This was corroborated by the observations of Capinera and Canoset (1979) that LC_{50} values between first and third instars of zebra caterpillar, Ceramica picta increased by 52 folds while the body weight increased by 56 folds.

An inverse relationship between LT_{50} values and the concentration of the virus was evident in the result. Even with the same concentration, prolongation in the mean time to death and LT_{50} values were noticed with advancement in the age of the test larvae. These were more distinct at lower doses of the virus than at higher doses. It is evident that at higher concentration of

the virus the disease initiated earlier and inflicted high mortality within a short time interval than at lower concentration.

The bioassay studies thus revealed the desirability of controlling the pest in the early stages of incidence since it will be possible to kill the early instars with low dosages of the virus and thus render the technology economically viable. The data also indicate the extent to which the doses in the field have to be regulated on the basis of the prevalence of different instars of larval population in the field at the time of control operation.

Apart from the infectivity and virulence of an insect pathogen, its performance in field will depend to a large extent on its stability under the changing environmental conditions. It must remain viable until it is infested by the target insect. Hence the information on the effective persistence of a candidate pathogen on the host plant was considered essential to assess its potential and to determine the required frequency of application in the field.

Nuclear polyhedrosis virus of O. arenosella applied on coconut foliage and exposed to weathering retained

substantial infectivity upto 84 hours after treatment. Then the virulence declined rapidly and there was complete inactivation in 168 hours. The half life of the pathogen on coconut foliage was 83.25 hours. Studies reported previously have shown varying degree of environmental stability of nuclear polyhedrosis virus. NPV of Heliothis when applied to cotton foliage lost most of its infectivity in one day (Bullock,1967). Ignoffo and Batzer (1971) observed 70 per cent loss in the infectivity of Heliothis virus in the first hour after application though 10 per cent of viral activity remained even after 192 hours of exposure. Gradual decline in pathogenicity resulting in complete inactivation in 10 days was reported in the case of NPV of L. fiscellaria somniaria and T. ni (Cunningham, 1970 and Jaques, 1971). The nuclear polyhedrosis virus of N. depunctalis retained substantial infectivity upto 98 hours of weathering on paddy foliage (Devanesan, 1979). The half life of the NPV of Heliothis on corn silk was estimated to be less than one day (Ignoffo et al., 1973) while its half life on soybean foliage was between 2 and 3 days (Ignoffo et al., 1974). The half life of S. mauritia NPV on paddy foliage was estimated to be 90.12 hours (Nair, 1981). The NPV of O. arenosella would

thus appear to be much more persistent than Heliothis virus while it is less persistent than the viruses of L. fiscellaria gemmiaria, T. ni, N. depunctalis and S. mauritia.

The effect of important factors which affect the viability of virus under field conditions were studied separately to understand the mechanism of the inactivation observed. When exposed to a constant temperature of 35°C, the infectivity declined rapidly beyond 84 hours of exposure. The half life of the virus was 87.10 hours only. Earlier authors also had reported that continuous exposure to higher range of temperature (35 to 40°C) affected viral stability and viral multiplication significantly (Bird, 1955; Thompson, 1959; Ignoffo, 1966b; Nair and Jacob, 1976; Pawar and Ramakrishnan, 1979). But Morris (1971) found that treating the NPV of L.fiscellaria gemmiaria at 45°C even for 200 hours did not affect its infectivity. The NPV of S. mauritia lost its infectivity only when treated for 168 hours at 35°C(Nair, 1981). The result of the present study showed that the NPV of O. arenosella is more thermostable than the other viruses studied except the NPV of L. fiscellaria gemmiaria and S. mauritia.

The NPV of O. arenosella retained substantial infectivity till 84 hours when exposed to infra red radiation. The half life of the virus was 85.22 hours. The loss of virulence by exposure to infra red rays was also evident from increase in LT_{50} values. Exposure of 105 minutes was sufficient to cause fifty per cent reduction in infectivity when the NPV of H. cunea was exposed to infra red radiation (Nordin, 1977). Exposure of the NPV of S. mauritia to infra red radiation showed that it retained substantial infectivity till 72 hours with an estimated half life of 75.34 hours (Nair, 1981). The results reveal that the NPV of O. arenosella is little more stable than the NPV of S. mauritia.

It was observed that exposure to UV rays emitted by the germicidal lamp caused a rapid loss in infectivity of the virus resulting in complete inactivation in 108 hours. The half life of the virus exposed to UV rays was 54.43 hours only. The loss of virulence by exposure to UV was evident from the increase in LT_{50} values also. The role of UV in reducing the pathogenicity of the insect viruses has been well documented. (Jaques, 1967a; David, 1969; Broome et al., 1974; Ignoffo et al., 1977; Witt and Stairs, 1977; Pawar and Ramakrishnan, 1979 and Nair, 1981). Ignoffo et al., (1977) found that the half life

of Heliothis NPV was 2.2 hours and Pawar and Ramakrishnan (1979) obtained complete inactivation of the NPV of S. litura by 15 minutes exposure to UV light.

Among the environmental factors studied in the present investigation UV radiation seems to be the most important one causing the inactivation of the NPV of O. arenosella. But Gaugler and Bousch (1978) observed that germicidal lamp emit rays of maximum effective range (253.7 nm) continuously and so the rate of inactivation of micro-organisms under such lamp occur more rapidly than it is possible under natural sunlight which has UV in the range of 291 to 380 nm. Hence he observed that laboratory results could not be related to field conditions. However, the observations made by David (1966), Jaques (1977), Ignoffo and Batzer (1971), Ignoffo et al., (1973), Young and Yearian (1974), Morris and Moore (1975) and Timans (1982) that the virus which was shielded or screened from UV rays in one way or other was more persistent, lend support to the view that UV radiation plays an important role in inactivating the virus in field.

Baculovirus are known to be effective in controlling many insect pests. In earlier days insect pathogens were considered to be safe and inoxious to other organisms.

But with the large scale introduction of microbial insecticides in the field of plant protection, elaborate safety tests became necessary.

Present studies showed that the NPV of O. arenosella has a wide host range even with interfamily infectivity. Safety to silk worm, Bombyx mori is of considerable importance in tracts with established sericulture industry and hence the pathogenicity/toxicity of the NPV of O. arenosella to the silk worm was studied in detail. Results showed that the pathogen did not cause any adverse effect on silk worms. All the treated larvae pupated normally and the adult emergence also was normal. The mean weights of cocoons from NPV fed and untreated lots of larvae were 1.560 g and 1.530 g respectively. Thus the virus was found to have no adverse effect on silk production too. Similar observations were made in the case of nuclear polyhedrosis virus of H. separata and Philosomia ricini (Dhaduti and Mathad, 1980) as well as the NPV of H. armigera (Narayanan, 1980).

O. arenosella is recognised as a pest amenable to biological control. Field release of laboratory bred larval parasitoids like B. brevicornis, P. nephantidis

and pupal parasitoids like T. pupivora. Brachymeria spp. are being regularly done for keeping the pest population under control. The safety of the virus to such parasitoids must be proved if the virus is to be included as a component in an integrated programme for controlling the pest. Hence safety tests were carried out with a larval parasitoid, B. brevicornis and a pupal parasitoid T. pupivora. The results showed that the parasitoids could develop even in the virus infected hosts and it did not directly or indirectly affect the development of the parasitoids, percentage of emergence, longevity and behaviour of the parasitoids. Similarly the virus fed parasitoids did not show any adverse effect on their fecundity, development and survival. Comparable results were obtained with the nuclear polyhedrosis virus of Heliothis by Narayanan (1980).

Effect of the virus on higher animals was studied by exposing white rats and white mice to massive doses of the pathogen. Body weight gain, temperature, and blood values of nuclear polyhedrosis virus fed animals were similar to those of untreated animals. The NPV treated animals did not show any abnormality or pathological conditions during the observation period of 21 days. Histological studies of the organs did not show

any evidence of tissue damage. Similar results were obtained for the nuclear polyhedrosis virus of H. armigera fed to white rats (Narayanan, 1979).

Embryonic tissues are known to be highly sensitive to microbial infection by virtue of low levels of antibodies in the tissues and also by virtue of the large number of actively dividing cells present. Hence embryonated chick eggs were exposed to the NPV of O. arenosella. The virus administered through different routes did not affect the growth of the embryo upto the end of the test period. There was no change in the general appearance of the embryo. No difference could be seen in the chorioallantoic membrane, allantoic fluid, amniotic fluid and yolk due to virus treatment. Post-mortem examination of the embryo did not show any pathological change in the tissues.

It is evident from the above results that the NPV of O. arenosella is safe to silk worm B. mori, parasitoids, B. brevicornis, T. pupivora, Vertebrates like white rat and white mice and even the highly sensitive chick embryo. A pathogen safe to the non-target organisms can hence be recommended for fighting the pest in field.

A preliminary field experiment was carried out for choosing a dose effective against the pest under field

conditions. The higher larval mortality of 85.00 per cent was recorded in plot treated with Thiodan 0.05 per cent spray and it was closely followed by NPV spray at 22.14 x 10^7 PIBs/ml with a larval mortality of 75.00 per cent there being no statistical difference between the two. Mortality in the control plot was 3.75 per cent only.

The results of the main field experiment showed that a substantial reduction in pest population was feasible with the NPV and the results were on par with Thiodan sprayed at 0.05 per cent concentration during the first week after treatment. The population build up in virus treated plot was slower than that of Thiodan treated plots. In the sixth week itself the population in Thiodan treated plot came on par with that of control while the population in NPV treated plot was still significantly lower. Even during the 14th week the population in NPV treated plot was significantly lower than that of Thiodan treated plot and in control. The prolonged effect observed in virus treated plot may indicate the establishment and self perpetuating trend of the pathogen among the host population. The same trend was observed in the results obtained from the three locations.

In an integrated control programme what is aimed at is the regulation of pest population below the level causing economic loss with least disruption to the natural balance. In the present instance the effectiveness of NPV of O. arenosella against the pest has been demonstrated by the comparatively high larval mortality. The experiment showed that the NPV at a dose of 22.14×10^7 PIBs/ml was as good as Thiodan 0.05 per cent spray for the control of O. arenosella in field. The slower rebuild of the pest population in virus treated plot indicated that a less frequent application of the microbial insecticides may be sufficient for the control of the pest. Frequent application of insecticides may lead to disruption of natural enemy complex and consequent resurgence of the pest. The hazards in applying the toxicants in the crown of trees and the possible environmental pollution also render the insecticides less preferable to the pathogen.

The incidence of parasitism in NPV and Thiodan treated plots did not vary significantly. The impact of the virus treatment which was found to be safe to the development and establishment of the natural enemies had to be reflected in the natural parasitoid population in virus treated plots in comparison with the population

in insecticide treated plots. Lack of significant data in this aspect of the experiment might be for want of repeated application of the toxicant and pathogen and due to the short span of time for which the observations were recorded. Lack of impact on the establishment of Compolatis chloridae parasitising H. armigera treated with an NPV was reported earlier also (Anon, 1983).

To sum up, the nuclear polyhedrosis virus of O. arenosella was found as a microbial insecticide highly infective to the pest and very virulent, invading almost all the important larval tissues in two to four days after inoculation. It showed the transovum transmission and thus revealed the possibility for self perpetuation in treated field populations of the pest. The virus was cross infective to six lepidopteran species. S. mauritia and S. litura which were more amenable to laboratory rearing were found to be more suitable for the mass multiplication of the NPV in laboratory. The virus was sufficiently persistent in the field. In low doses, the pathogen controlled the first three larval instars of O. arenosella effectively and hence the treatment given at the beginning of the pest incidence will provide an economically viable and

effective control strategy for combating the pest. The pathogen was found to be very safe to the non-target organisms. The virus will thus admirably fit in an integrated control strategy against O. arenosella. Being cross transmissible to other important crop pests, the possibility of developing the pathogen as a broad spectrum insecticide also exists.

SUMMARY

SUMMARY

Detailed studies were carried out on the nuclear polyhedrosis virus of O. arenosella covering symptomatology, morphology of the pathogen, host-pathogen relationship, mode of transmission to the progeny, bioassay of viral activity, cross transmission to other species of lepidoptera, persistence in field, effect of various environmental factors on persistence, safety to non-target organisms and field efficacy of the virus in controlling the pest.

For studying the symptomatology of the disease, different larval instars were exposed to the virus adopting spot feeding technique and the infected larvae were reared in the laboratory. The symptoms noted were broadly similar to those reported as typical for the nuclear polyhedoris of lepidopteran larvae. Three to four days after ingestion of the virus, the larvae became paler and lethargic. They showed loss of appetite. Just prior to death, feeding and movements ceased completely. The cuticle became fragile and body tissues got liquefied and milky white in colour. Even slight disturbances by other moving larvae caused the rupture of the body wall and the release of polyhedral bodies. The cadavers

were found sticking to the leaf blade which became deeply darkened in 24 to 48 hours. Some of the diseased pupae exhibited typical symptom of larval pupal mosaic. Malformed adults with short and ruffled wings also emerged. The body tissues and body fluids of those adults contained large number of polyhedral inclusion bodies.

The electron micrography of the virus was got down by NERC Institute of Virology, Oxford, England. The polyhedra of nuclear polyhedrosis virus of O. arenosella were irregular in shape and the surface was smooth without any ornamentation. The diameter ranged from 533.33 nm to 1666.67 nm with an average of 1393.30 nm. The virus was 'multiple embedded' since a number of rods were enclosed in one developmental membrane.

The histopathological studies were done on third instar larvae killed at 24 hour intervals upto 144 hours after inoculation. Azan staining technique was adopted for the purpose. No polyhedral bodies were visible in any of the tissues at 24 hours after inoculation. At 48 hours after inoculation the nuclei of hypodermis, fat body, trachea, connective tissues surrounding the gut and the gut epithelium were seen hypertrophied and got stained.

The infection progressed further by 72 hours after inoculation in the above tissues. In addition to the above tissues Malpighian tubules, nerve ganglia and muscles were also seen infected. Fully developed polyhedra were visible in the nuclei of the above tissues by 96 hours after inoculation. At this stage many of the cells of the epicholial sheath of gonads also showed signs of infection. At 120 hours after inoculation the infection had spread completely over the cells of hypodermis, fat body, trachea and epithelial sheath of gonads and by 144 hours after inoculation many tissues showed signs of disintegration and polyhedra were seen released into the haemocoel. No infection in the tissues of silkgland, brain, pericardial cells and wing buds could be detected. Thus the nuclear polyhedrosis virus of O. arenosella was found to be a polyorganotrophic virus and extensive invasions of the larval tissues by the pathogen indicated its high virulence.

Early fifth instar larvae fed with sublethal doses of the virus pupated and emerged as moths. The eggs laid by the moths caused significantly high mortality among the emerging larvae while the surface sterilization of eggs deprived them of the infectivity. The homogenates of the above two lots of eggs when fed to third instar larvae of

O. arenosella also showed the same results. These results showed the occurrence of transovum transmission of the virus in O. arenosella. When gravid moths were fed with virus contaminated honey or when the external genitalia were smeared with the virus, the disease was transmitted to the next generation through the eggs. Such eggs when surface sterilized lost the infectivity. These results also endorsed the possibility of transovum transmission of the virus by the infected or contaminated moths. The presence of the virus in the sheath of the gonads seen in histopathological studies also indicated the transmission of the virus through the eggs. The moths of O. arenosella thus serve as effective carriers of the pathogen and help in the natural perpetuation and spread of the disease.

Cross transmission studies of nuclear polyhedrosis virus of O. arenosella to ten other species of Lepidoptera were carried out and the virus was found infective, to the noctuids S. litura, S. mauritia, A. ceponis and arctiids P. ricini and D. obliqua. The recovery of polyhedra was higher in S. litura and S. mauritia. Those insects which were more amenable to laboratory rearing also were chosen as suitable alternate hosts for large scale multiplication of nuclear polyhedrosis virus of O. arenosella in the

laboratory. The repeated multiplication of the virus in alternate hosts neither reduced its infectivity to O. arenosella nor its virulence. Results also indicated the possibility of using the virus as a broad spectrum microbial insecticide against all the pests which were found susceptible to the pathogen.

To find out the optimum dose of the pathogen and the more susceptible stage of the pest for evolving a proper technology for the control of the pest in field, bioassay of the viral activity was done using the different larval instars of O. arenosella. The LC_{50} and LT_{50} values increased with the age of the host. The LC_{50} values ranged from 9.247 PIBs/ml for the first instar to 33775.361 PIBs/ml for the fifth instar larvae. The results indicated a progressive decrease in susceptibility to the virus as the larvae advanced in age. It revealed the desirability of applying the virus in the early stages of pest incidence in field since it is possible to kill the earlier instars with low dosages of the pathogen thus rendering the technology economically viable.

The stability of the pathogen under field conditions was studied in detail. The virus was applied on coconut foliage and it was exposed to weathering. Samples were

drawn at 12 hour intervals upto 168 hours after spraying. The results showed that the virus retained substantial infectivity upto 84 hours after spraying and thereafter a gradual decline in its virulence was noticed leading to complete inactivation at 168 hours. The LT_{50} values also increased with the increase in the duration of weathering.

The virus was exposed to the different components of the environment viz high temperature, infrared radiations and ultraviolet rays to understand the mechanism of inactivation of the virus.

The half life of the virus was found to be 83.25 hours when subjected to weathering on plant foliage, 87.10 hours when heated at 35°C continuously, 85.22 hours when exposed to infrared rays and 54.43 hours when exposed to ultraviolet rays. The most rapid inactivation was caused by ultraviolet rays followed by infrared rays and heat treatment.

The pathogenicity/toxicity of nuclear polyhedrosis virus of *O. arenosella* on silkworm, *Bombyx mori* was studied extensively. The virus had no adverse effect on the survival and development of silkworm or production of silk.

O. arenosella is recognised as a pest amenable to biological control and hence safety tests to a larval parasitoid B. brevicornis and a pupal parasitoid T. pupivora were carried out and the results showed that the virus neither directly or indirectly affected the development of the parasitoids nor their activity.

The effect of virus on higher animals (white rats and white mice) was studied and the results showed that the virus neither infected the animals nor did it cause any abnormality in their physiology and behaviour. Histological studies of different organs did not show any evidence of tissue damage.

The effect of the virus on tissues of developing chick embryo was studied. The virus was administered through four different routes into the embryonated chick egg and the results showed that the virus did not affect the development of the embryo or its growing tissues. Thus the nuclear polyhedrosis virus of O. arenosella was found to be safe to silkworm, parasitoids, vertebrates and even the developing chick embryo.

A preliminary field experiment was carried out for choosing a dose effective against the pest under field conditions. The results of the experiment showed that

22.14 x 10^7 PIBs/ml was on par with Thiodan 0.05 per cent in causing the mortality of the larvae of O. arenosella.

When the above dose of nuclear polyhedrosis virus was compared with Thiodan 0.05 per cent in a field experiment, done at three locations, both treatments were on par in reducing the pest population as observed one week after the treatment. At the seventh week the population of the pest in nuclear polyhedrosis virus treated plots was much lower than that of the Thiodan treated plots while the latter came on par with the control. Thus in the long run, the nuclear polyhedrosis virus was more effective than the insecticide in regulating the pest population. The prolonged effect observed in virus treated plots indicated the possible establishment and self perpetuating trend of the pathogen in the host population.

The incidence of parasitism in nuclear polyhedrosis virus and Thiodan treated plots did not vary significantly probably because of the short duration of observations. The nuclear polyhedrosis virus of O. arenosella was thus found as a microbial insecticide effective in controlling the pest and it was found safe to non-target organisms. This can be developed as an effective component of an integrated control programme for the control of O.arenosella.

REFERENCES

REFERENCES

Abul-Nasr, S. (1959). Further tests on the use of polyhedrosis virus in the control of the cotton leaf worm, Prodenia litura F. J. Insect Pathol. 1: 112-120.

Adams, J.R. and Wilcox, T.A. (1968). Histopathology of the Almond moth, Cadra cautella infected with a nuclear polyhedrosis virus. J. Invertebr.Pathol. 12: 269-274.

Adams, J.R., Wallis, R.L., Wilcox, T.A. and Faust, R.M. (1968). A previously undescribed polyhedrosis of the zebra caterpillar, Ceramica picta. J. Invertebr. Pathol. 11: 45-48.

Aizawa, K. (1953). On the activation of silkworm jaundice virus. Jap. J. appl. Ent. Zool. 17: 181-190.

Aizawa, K. (1955). Inactivation of silkworm jaundice virus by ultraviolet irradiation. J. Sericult. Sci. Japan. 24: 398-399.

Aizawa, K. (1962). Infection of greater wax moth Galleria mellonella (Linn.) with nuclear polyhedrosis of silkworm. J. Insect Pathol. 4: 122-127.

Aizawa, K. (1963). The nature of infection caused by nuclear polyhedrosis viruses. In "Insect Pathology An Advanced Treatise" (E.A.Steinhaus, ed.) Vol. I. pp. 381-412. Academic Press, New York.

Allen, G.E. and Ignoffo, C.M. (1969). Nuclear polyhedrosis of Heliothis. Quantitative in vivo estimation of virulence. J. Invertebr. Pathol. 13: 378-381.

Allen, G.E., Gregory, B.G. and Pate, T.L. (1967). Field evaluation of nuclear polyhedrosis virus in the control of Heliothis zea and H. virescens in cotton. J. Invertebr. Pathol. 9: 32-40.

Andrews, G.L. and Sikorowski, P.P. (1973). Effect of cotton leaf surfaces on the nuclear polyhedrosis virus of Heliothis zea and Heliothis virescens (Lepidoptera: Noctuidae) J. Invertebr. Pathol. 22: 290-291.

Anonymous, 1983. Proceedings of the Fourth All India Workshop on Biological control. Bangalore, India 22nd to 24th Feb. 1983.

Aratake, Y. and Kayamura, T. (1972). Cross-transmission of polyhedrosis virus of gypsy moth Lymantria dispar japonica Motschulsky, to the silkworm Bombyx mori and other lepidopterous pests. Proceedings of the Association of Plant Protection of Kyushu. 18: 17-20.

Aratake, Y. and Kayamura, T. (1973). Pathogenicity of a nuclear polyhedrosis virus of the silkworm, Bombyx mori for number of lepidopterous insects. Jap. J. appl. Entomol. Zool. 17: 121-126.

Arugua, H., Fukuda, C. and Joshitake, N. (1963). Observations of polyhedrosis within nucleus of the silkgland cell of the silkworm, Bombyx mori, L. Nippon. Sashicuku Zasshi. 32: 213-218.

Balch, R.E. and Bird, F.T. (1944). A disease of the European spruce sawfly Gilpinia hercyniae (Hlg). and its place in natural control. Sci. Agri. 25: 65-80.

Beegle, C.C. and Oatman, E.R. (1975). Effect of a nuclear polyhedrosis virus on the relationship between Trichoplusia ni (Lepidoptera: Noctuidae) and the parasite, Hyposoter exiguae (Hymenoptera: Ichneumonidae) J. Invertebr. Pathol. 25(1): 59-71.

Benz, G. (1963). A nuclear polyhedrosis of Malacosoma alpicola (Staudinger). J. Insect Pathol. 5: 215-241.

Bergerjon, A., Blache, G., Chaufaux, J. and Petre, Z. (1981). Comparative susceptibility as a function of age of larvae of Lymantria dispar, Mamestra brassicae and Spodoptera littoralis to the nuclear polyhedrosis virus. Entomophaga 26(1): 47-58.

*Bergold, G.H. (1943). Uber Polyeder Krunheiten Boi. Insekten. Biol. Zbl. 63: 1-55.

Bergold, G.H. (1953). Insect viruses. Adv. virus Res. 1: 99-139.

Bergold, G.H. (1963). The nature of nuclear-polyhedrosis viruses "In Insect Pathology an advanced treatise" (E.A.Steinhaus, ed) Vol. 1. pp.413-456. Academic Press, New York.

Bergold, G.H. and Ripper, W.E. (1957). The polyhedral virus of Heliothis armigera (Hbn). Nature. Lond. 180: 764-765.

Bird, F.T. (1953). The use of a virus disease in the biological control of the European pine sawfly. Neodiprion sertifer (Geoff). Can Ent. 85: 437-446.

Bird, F.T. (1955). Virus disease of sawflies. Can Ent. 87: 432-436.

Bird, F.T. (1961). Transmission of some insect viruses with particular reference to ovarial transmission and its importance in development of epizootics. J. Insect Pathol. 3: 352-380.

Bird, F.T. and Burk, J.M. (1961). Artificially disseminated virus as a factor controlling the European spruce sawfly, Diprion hercyniae (Htg.) in the absence of introduced parasites. Can Ent. 93: 228-238.

Bird, F.T. and Whalen, M.M. (1953). A virus disease of the European pine sawfly Neodiprion sertifer. Can. Ent. 85: 433-437.

*Bolle, J. (1908). Z. Landwirtsch. Versuchsu Oesterr. 11: 279-280.

Boucias, D.G., Johnson, D.W. and Allen, G.E. (1980). Effect of host age, virus dosage and temperature on the infectivity of a nuclear polyhedrosis virus against velvet bean caterpillar, Anticarsia gemmatalis larvae. Environ. Entomol. 9(1): 59-61.

Broome, J.R., Sikorowski, P.P. and Neal, J.W. (1974). Effect of sunlight on the activity of nuclear polyhedrosis virus from Malacosoma disstria. J. Econ. ent. 67: 135-136.

Brown, E.S. and Swaine, G. (1965). Virus disease of the African armyworm. Spodoptera exempta (Wlk.) Bull. Ent. Res. 56: 95-116.

Bullock, H.R. (1967). Persistence of Heliothis nuclear polyhedrosis virus on cotton foliage. J. Invertebr. Pathol. 9: 432-434.

Bullock, H.R., Hollingworth, J.R. and Hartstack, A.W.Jr. (1970). Virulence of Heliothis nuclear polyhedrosis virus exposed to monochromatic irradiation. J. Invertebr. Pathol. 16: 419-422.

Cantwell, G.E. (1967). Inactivation of biological insecticides by irradiation. J. Invertebr. Pathol. 9: 138-140.

Capinera, J.L. and Canoset, R.L. (1979). Susceptibility of zebra caterpillar to Autographa californica polyhedrosis virus J. Econ. Ent. 72: 570-572.

Chan, M.-S., Sum, P.L. and Xu, L.H. (1981). Study of the biological characteristics of Nuclear polyhedral virus of Buzura suppressaria Guenec. Nubu. Nongye Kexue. 12: 26-28.

Chapman, A.J. and Dell, R.A. (1967). Field tests with NPV for the control of Heliothis spp. in the southern high planes of Texas. J. Invertebr. Pathol. 9: 434-436.

Clark, E.C. (1956). Survival and transmission of a virus causing Polyhedrosis in Malacosoma fragile. Ecology 37: 728-732.

Clark, E.C. and Thompson, C.G. (1954). The possible use of microorganisms in the control of the great basin tent caterpillar. J. Econ. Ent. 47: 268-272.

*Conte, A. (1907). Compt. Rendsoession Assoc.France. Advance Sci. 36: 622-623.

Creighton, C.S., McFadden, E.L., and Cuthbert, R.B. (1971). The use of nuclear polynedrosis virus of Heliothis in the control of tomato fruit borer. J. Econ. Ent. 64: 737-739.

Cunningham, J.C. (1970). Persistence of the nuclear polyhedrosis of the eastern hemlock looper on balsam foliage. Bi-montnly Research Notes 26: 24-25.

Cunningham, J.C. (1971). An ultrastructural study of the development of a nuclear polyhedrosis virus of the eastern hemlock looper, Lambdina fiscellaria fiscellaria Can J. Microbiology 17: 69-72.

Cunningham, J.C. and Entwistle, P.F. (1981). Control of Sawflies by baculovirus In 'Microbial Control of Pests and Plant diseases 1970-1980. (Burges, H.D. ed.) pp. 379-408. Academic Press, London.

David, W.A.L. (1966). Factors influencing the persistence of granulosis virus of Pieris brassicae in the environment. Proc. Int. Coll. Insect Pathology. Wageningen, Netherlands, 1966, pp. 175-178.

David, W.A.L. (1969). Effect of Ultraviolet radiation of known wavelength on a granulosis virus of Pieris brassicae. J. Invertebr. Pathol. 14: 336-342.

David, W.A.L. and Magnus, L.A. (1967). Preliminary observations in the in vitro photo-reactivation on an insect virus inactivated with ultraviolet radiation. J. Invertebr. Pathol. 9: 266-268.

Davanesan, S. (1979). Studies on nuclear polyhedrosis virus of rice case worm, Nymphula depunctalis Guen (Pyraustidae: Lepidoptera). M.Sc.(Ag). thesis, Kerala Agricultural University, Vellanikkara.

Dhaduti, S.G. and Mathad, S.B. (1980). Effect of nuclear polyhedrosis virus of the armyworm, Mythimna separata on colonies of Apis cerana indica. J. Apic. Res. 19(1): 77-78.

Doane, C.C. (1967). Bioassay of nuclear polyhedrosis virus against larval instars of gypsy moth. J. Invertebr. Pathol. 9: 376-386.

Doane, C.C. (1969). Transovum transmission of a nuclear polyhedrosis virus in gypsy moth and the inducement of virus susceptibility. J. Invertebr. Pathol. 14: 199-210.

Doller, G. and Growner, A. (1981). Safety study for testing virus replications of the nuclear polyhedrosis virus of Mamestra brassicae in vertebrates. Z. angew. Ent. 92(1): 99-105.

Drake, E.L. and McEwen, F.L. (1959). Pathology of a nuclear polyhedrosis of the cabbage looper, Trichoplusia ni (Hubner) J. Insect Pathol. 1: 281-293.

Elgee, E. (1975). Persistence of a virus of the white marked tussock moth on balsam fir foliage. Bimonthly Research Notes 31: 33-34.

Elmore, J.C. and Howland, A.F. (1964). Natural and artificial dissemination of nuclear polyhedrosis virus by contaminated adult cabbage loopers. J. Insect Pathol 6: 430-438.

Elnagar, S. and Abul-Nasar, S. (1980). Effect of direct sunlight on the virulence of nuclear polyhedrosis virus of the cotton leaf worm Spodoptera littoralis Z. angew. Ent. 90(1): 75-80.

Entwistle, P.F. and Adams, P.H.W. (1977). Prolonged retention of infectivity of the NPV of Gilpinia hercyniae (Hymenoptera: Diprionidae) on foliage of spruce species. J. Invertebr. Pathol. 8: 145-149.

Evans, H.F. (1981). Quantitative assessment of the relationship between dosage and response of the nuclear polyhedrosis virus of Mamestra brassicae J. Invertebr. Pathol. 37(1): 101-109.

Falcon, L.A. (1973). Biological factors that affect the success of microbial insecticides development or integrated control Regulation of insect populations by microorganisms. Ann. N.Y. Acad. Sci. 217: 173-186.

Falcon, L.A., Leigh, T.F., Van Den Bosch, R., Black, J.H. and Burton, B.S. (1965). Insect diseases tested for control of cotton bollworm. Calif. Agric. 19: 12-14.

Finney, D.J. (1952). Probit analysis - A Statistical Treatment of the Sigmoid response curve. Second edition, University Press, Cambridge, pp.318.

Fraser, M.J. and Stairs, G.R. (1982). Susceptibility of Trichoplusia ni, Heliothis zea (Noctuidae) and Manduca sextra (Sphingidae) to a nuclear polyhedrosis virus from Galleria mellonella (Gallerildae) J. Invertebr Pathol. 40(2): 255-259.

Gaugler, R. and Bousch, G.M. (1978). Effect of UV radiation and sunlight on the entomogenous nematode, Neoaplectana carpocapsae J. Invertebr. Pathol. 32: 291-294.

George, M.V., Santhamma, B and Vijayakumar, K. (1982). A Technique to ostimate the field population of Nephantis serinopa a pest of coconut palm. Placrosym V. 5th Annual Symposium on Plantation Crops. Dec. 15-18, 1982.

Gerhenson, S.M. (1960). A study on a mutant strain of nuclear polyhedral virus of Oak silkworm. Probl. Virol (USSR) (English Transl.) 6: 720-725.

Getzin, L.W. (1962). The effectiveness of nuclear polyhedrosis virus for control of cabbage looper. Trichoplusia ni. J. Econ. Ent. 55: 442-445.

Glaser, R.W. (1928). Virus diseases of insects. In "Filterable viruses" (Rivers, T.M. ed) pp.301-333. Williams and Wilkins, Baltimore.

Gudawskas, R.S. and Canerday, D. (1968). The effect of heat buffer sale and H ion concentration and ultraviolet light on the infectivity of Heliothis and Trichoplusia nuclear polyhedrosis viruses. J. Invertebr Pathol. 12: 405-411.

Hafez, M. (1958). Studies on the polyhedrosis virus disease of the cotton leaf worm, Prodenia litura F. in Egypt. Bull Soc. ent. Egypt. 42: 357-570.

Hall, I.M. (1957). Use of polyhedrosis virus to control cabbage looper on lettuce in California J. econ. Ent. 54: 921-923.

Hamm, J.J. (1966). A modified azan staining technique for inclusion body viruses. J. Invertebr. Pathol. 8: 125-126.

Hamm, J.J. (1968). Comparative histopathology of a granulosis and a nuclear polyhedrosis virus of Spodoptera frugiperda J. Invertebr. Pathol. 10: 320-326.

Hamm, J.J. and Young, J.R. (1974). Mode of transmission of a nuclear polyhedrosis virus to progeny of adult Heliothis zea. J. Invertebr. Pathol. 24: 70-81.

Harpaz, I. and Ben-Shaked, Y. (1964). Generation to generation transmission of the NPV of Prodenia litura (Fabricius) J. Insect Pathol. 6: 127-130.

Harpaz, I. and Raccah, B. (1976). Nuclear polyhedrosis of Egyptian cotton work Spodoptera littoralis (Lepidoptera: Noctuidae) Temperature, pH reactions host range and synergism. J. Invertebr. Pathol. 32: 368-372.

Harpaz, I. and Zlotkin, E. (1965). A nuclear polyhedrosis virus of the safflower leaf worm, Heliothis peltigera schiff (Lepidoptera: Noctuidae) Annls. Soc. ent. Fr. 1: 963-972.

Harper, J.D. (1976). Cross infectivity of six plusiine nuclear polyhedrosis virus isolates to plusiine hosts. J. Invertebr. Pathol. 27: 275-277.

Hassan, S.A. and Groner, A. (1977). The effect of the nuclear polyhedrosis virus (Baculovirus sp.) of Mamestra brassicae on Trichogramma cacoecia (Hym: Trichogrammatidae) and Chrysopa carnea (Neur: Chrysopidae) Entomophaga 22(37): 281-288.

Heimpel, A.M. (1966). The nuclear polyhedrosis virus of Trichoplusia ni. I. Toxicity/ Pathogenicity of virus to white rats and guinea pigs. J. Invertebr. Pathol. 7: 342-345.

Heimpel, A.M. and Adams, J.R. (1966). A nuclear polyhedrosis virus of cabbage looper, Trichoplusia ni. J. Invertebr. Pathol. 8: 340-346.

Henneberry, T.T. and Kishaba, A.N. (1966). Cabbage looper, in "Insect colonisation and mass production", (C.N.Smith, ed) pp. 461-478. Academic Press, New York.

Hirt, R.C., Schmit, R.G., Seesle, N.O. and Sullivan, A.P. (1960). Ultraviolet spectral energy distribution of natural sunlight and accelerated test light sources, J. Opt. Soc. Amer. 50: 706-713.

Hoffmaster, R. and Pitman, L.P. (1961). Utilization of a nuclear polyhedrosis virus to control the cabbage looper on cole crops in virgina. J. econ. Ent. 54: 921-923.

Hoskins, J.M. (1967). Embryonated egg technique. In "Virological procedures". Butterworth & Co. (Publishers) Ltd. pp.93-101.

Hostetter, K.D., Biever, K.D., Heimpel, A.M. and Ignoffo, C.M. (1979). The efficacy of the nuclear polyhedrosis virus of the alfalfa looper against cabbage looper larvae on cabbage in Missouri. J. econ. Ent. 12: 93-97.

Hunter, D.K. and Hall, I.M. (1968). Cytopathology of nucleopolyhedrosis of the beet army worm, Spodoptera exigua. J. Invertebr. Pathol. 12: 93-97.

Hunter, D.K., Hoffman, D.F. and Collier, S.J. (1973). Cross infection of a nuclear polyhedrosis virus of almond moth to the Indian meal moth. J. Invertebr. Pathol. 20: 186-192.

Hwang, G.H. and Ling, T. (1975). Studies on the nuclear polyhedrosis virus disease of the cotton leaf worm, Prodenia litura. F. Acta Ent. sinca. 18: 17-24.

Ignoffo, C.M. (1965a). The nuclear polyhedrosis virus of Heliothis zea (Boddie) and Heliothis virescens (Fabricius) i. Virus propagation and its virulence. J. Invertebr.Pathol. 7: 209-216.

Ignoffo, C.M. (1965b). The nuclear polyhedrosis virus of Heliothis zea (Boddie) and Heliothis virescens (Fabricius) iv. Bioassay of viral activity. J. Invertebr. Pathol. 7: 315-319.

Ignoffo, C.M. (1966a). Insect viruses. In "Insect colonization and mass production (C.N.Smith, ed.) pp. 501-530. Academic Press, New York.

Ignoffo, C.M. (1966b). Effect of temperature on mortality of Heliothis zea larvae exposed to sublethal doses of a nuclear polyhedrosis virus. J. Invertebr. Pathol. 8: 390-292.

Ignoffo, C.M. (1968). Viruses- Living Insecticides. In "Current topics in Microbiology and Immunology" (K.Maramorosch, ed.) Vol. 42, pp. 129-167. Springer Verlag, Berling, Middleberg, New York.

Ignoffo, C.M. and Batzer, C.F. (1971). Micro encapsulation and ultraviolet protectant to increase sunlight stability of an insect virus. J. econ. Ent. 64: 850-853.

Ignoffo, C.M. and Heimpel, A.M. (1965). The nuclear polyhedrosis virus of Heliothis zea (Boddie.) and Heliothis virescens (Fabricius) V.Toxicity. Pathogenicity of virus to white rats and guinea pigs. J. Invertebr. Pathol. 7: 329-340.

Ignoffo, C.M. Chapman, A.J. and Martin, D.F. (1965). A nuclear polyhedrosis virus of Heliothis zea (Boddie.) and Heliothis virescens (F.) III Effectiveness of the virus against field population of Heliothis on cotton, corn and grain sorghum. J. Invertebr. Pathol. 7: 227-235.

Ignoffo, C.M. Hostetter, D.L. and Pinnel, R.E. (1974). Stability of Bacillus thuringiensis and Baculovirus heliothis on soybean foliage. Environ. Entomol. 3: 117-119.

Ignoffo, C.M., Parker, F.D., Boening, O.P., Pinnol, R.E. and Hostetter, D.L. (1973). Field stability of Heliothis Nuclear polyhedrosis virus on corn silks. Environ Entomol. 2: 302-303.

Ignoffo, C.M. Hostetter, D.L., Sikorowski, P.P., Sutter, G. and Brooks, W.M. (1977). Inactivation of representative species of entomopathogenic virus, a bacterium, a fungus and protozoa by ultraviolet light source. Environ. Entomol. 6: 411-415.

Injac, M., Duthiot, J.L. and Amaragier, A. (1973). Histopathological and cytopathological study of a nuclear polyhedrosis of the fall web worm Hypantria cunea (Lepidoptera: Arctiidae) Annale Zool. Ecol. Animale. 5(1): 95-110.

Jacob, A. (1972). Studies on nuclear polyhedrosis of three species of Lepidoptera. Ph.D. thesis. Tamil Nadu Agricultural University, Coimbatore.

Jacob, A. and Subramaniam, T.R. (1972a). Effect of larval age and dosage of virus on the susceptibility of Spodoptera litura F. to a nuclear polyhedrosis. Agric. Res. J. Kerala 10: 176-178.

Jacob, A. and Subramaniam, T.R. (1972b). Nuclear polyhedrosis of some Lepidoptera. Curr. Sci. 41: 536.

Jacob, A. and Subramaniam, T.R. (1973). Histopathology of tobacco caterpillar, Spodoptera litura F. infected with a nuclear polyhedrosis virus. Agric. Res. J. Kerala 11: 114-118.

Jacob, A. and Subramaniam, T.R. (1974). Influence of nuclear polyhedrosis on larval growth, moulting and food consumption of Spodoptera litura. Madras Agric. J. 61: 189-192.

Jacob, A. and Thomas, M.J. (1972). A. Nuclear polyhedrosis of Diacrisia obliqua (Wlk.)(Arctiidae Lepidoptera) Agric. Res. J. Kerala 10: 182.

Jacob, A., Saradamma, K. and Thomas, M.J. (1973). A nuclear polyhedrosis of the rice swarming caterpillar Spodoptera mauritia Boisduval: (Lepidoptera: Noctuidae) Curr. Sci. 42: 369.

Jacquemard, P. (1978). Pathogenic action of the nuclear polyhedrosis virus of Mamestra brassicae on Diparopsis watersi (Lepidoptera: Noctuidae) Coton et Fibres Tropicales 33(2): 307-308.

Jacquemard, P. and Delattre, R. (1977). Pathogenic action of the nuclear polyhedrosis virus of Autographa Californica on Diparopsis watersi (Lepidoptera: Noctuidae) Coton et Fibres Tropicales 32(3): 249-252.

Jaques, R.P. (1957). The persistence of nuclear polyhedrosis virus in the habitat of the host insect. Trichoplusia ni 1. Polyhedra on cotton foliage. Can Ent. 99: 785-794.

Jaques, R.P. (1971). Tests on protectants for foliar deposits of polyhedrosis virus. J. Invertebr. Pathol. 17: 9-16.

Jaques, R.P. (1975). In Baculoviruses for Insect pest control (Summers, M.D. et al., eds.) pp. 90-101. Am. Soc. Microbiol. Publ. Washington.

Jaques, R.P. (1977). Field efficacy of viruses infections to imported cabbage worm on late cabbage. J. econ. Ent. 70: 111-118.

Kaya, H.K. (1977). Transmission of a nuclear polyhedrosis virus isolated from Autographa californica to Alsophila pometria. Hyphantria cunea and other forest defoliators. J. econ. Ent. 70(1): 9-12.

Kinzer, R.E., Bariola, L.A., Ridgway, R.L. and Jones, S.L. (1976). Systemic insecticides and NPV for the control of the bollworm and tobacco budworm on cotton. J. econ. Ent. 69: 697-701.

Kunimi, Y. (1982). Transovum transmission of a nuclear polyhedrosis virus of the fall webworm, Hyphantria cunea Drury (Lep: Arctiidae). Appl.Ent. Zool. 17 (3): 410-417.

Lathika, P. and Jacob A. (1974a). Investigations on a nuclear polyhedrosis of Spodoptera mauritia (Boisduval) (Noctuidae: Lepidoptera) infected with a nuclear polyhedrosis virus. Agric. Res. J. Kerala. 12: 1-6.

Lathika, P. and Jacob, A.(1974b). Changes in haemocyte counts in larvae of Spodoptera mauritia (Boisduval) infected with a nuclear polyhedrosis virus. Agric. Res. J. Kerala. 12: 91-94.

Lathika, P. and Jacob, A. (1974c). Effect of temperature and sunlight on the infectivity of a nuclear polyhedrosis virus of Spodoptera mauritia (Boisduval) Curr.Sci. 43: 587-588.

Larionov. G.V. and Bakhalov, L.A.(1974). On the question of transovariel transmission of the nuclear polyhedrosis virus of the nun moth. Ser Biol. Nouk. 15(3): 60-65.

* Laudeho, Y and Amargier,A. (1963). Virose a polyhedres nucleaires a localisation inhabituelle chezun Lepiodoptera. Rev. Pathol. Vegetab. Entomol. Agri. France 42: 207-210.

Lautenschlager, R.A. and Podgwaite, J.D. (1977). Passage of infectious nuclear polyhedrosis virus through the alimentary tract of two small mamel predators of the gypsy moth, Lymantria dispar. Environ Entomol. 6(5): 737-738.

Lautenschlager, R.A., Kircher, C.H. and Podgwaite, J.D. (1977). Effect of nuclear polyhedrosis virus on selected mammalian predators of the gypsy moth. Forest Service Research paper, USDA. 6: 377.

Lautenschlager, R.A., Podgwaite, J.D. and Watson, D.E. (1980). Natural occurrence of the NPV of the gypsy moth Lymantria dispar (Lepidoptera-Lymantridae) in wild birds and mamels. Entomophaga. 25(3): 261-267.

Legacion, D.M. and Gabriel, B.P.(1978). Dosage-mortality study on the nuclear polyhedrosis of the common cut-worm, Spodoptera litura Fab. (Lepidoptera: Noctuidae) Philippines Entomologist: 3(2): 109-116.

Lewis, F.B. (1960). How to collect and process small polyhedral viruses of Insects. Forest Research Notes, Northeastern Forest Experimental Station No.109: 1-8.

Lewis, F.B. (1981). Control of Gypsy moth by a baculovirus In "Microbial control of pests and plant diseases 1970-1980" (Burges, H.D. ed.) pp: 363-378. Academic Press, London.

Lewis, F.B., Lynch, R.E. and Jackson, J.J. (1977). Pathology of a baculovirus of the alfalfa looper, Autographa californica in the European Corn borer. Ostrinia nubilalis Environ. Entomol. 6(4): 535-538.

Livingston, J.M. and Yearian, W.C. (1972). A nuclear polyhedrosis virus of Pseudoplusia includens (Lep: Noctuidae). J. Invertebr. Pathol. 19: 107-112.

Magnoler, A. (1974). Bioassay of a nuclear polyhedrosis virus of the gypsy moth, Porthetria dispar L. J.Invertebr. Pathol. 23: 190-196.

Magnoler, A. (1975). Bioassay of polyhedrosis virus against larval instars of Malacosoma neustria L. J. Invertebr. Pathol. 25: 343-348.

Manjunath, D. and Mathad, S.B. (1978). Temperature tolerance, thermal inactivation and ultraviolet resistance of nuclear polyhedrosis virus of the army worm Mythimna separata (Wlk). (Lep: Noctuidae).Z. angew. Ent. 87: 82-90.

Martignoni, M.E. (1964). Pathophysiology in the insect. Ann. Rev. Ent. 9: 179-206.

Martignoni, M.E. and Iwai, P.J. (1977). Thermal inactivation characteristics of two strains of nucleopolyhedrosis virus (Baculovirus sub group A) pathogenic for Orgyia pseudotsugata. J. Invertebr. Pathol. 30: 255-262.

Martignoni, M.E. and Milstead, J.E. (1962). Transovum transmission of the NPV of Colias eurytheme (Boisd.) through contamination of the female genitalia. J. Insect Pathol. 4: 113-121.

Mathad, S.B., Splittstoesser, C.M. and McEwen, F.L. (1968). Histopathology of the cabbage looper, Trichoplusia ni infected with a nuclear polyhedrosis. J. Invertebr. Pathol. 11: 456-464.

Mathew, P.M. (1980). Survey of the microbial diseases of important vegetable pests of Kerala and detailed studies on the pathogens affecting snakegourd semilooper. Anadevidia peponis Fabricius. (Noctuidae: Lepidoptera). M.Sc.(Ag) thesis, Kerala Agricultural University, Vellanikkara.

McCollom, G.B. and Reed, E.M. (1974). A nuclear polyhedrosis virus of the light brown apple moth, Epiphyas postvittana J. Invertebr. Pathol. 18(3): 337-343.

McEwen, F.L. and Hervey, G.E.R.G.(1958). Control of cabbage looper with a virus disease. J. econ. Ent. 51: 626-631.

McGarr, R.L. (1968). Field test with a nuclear polyhedral virus against the bollworm and tobacco budworm. J.econ. Ent. 61: 342.

McGarr, R.L. and Ignoffo, C.M. (1966). Control of Heliothis sp. with a NPV, CPV and two newer insecticides, J. econ. Ent. 59: 1284-1285.

McLeod, P.J., Yearian, W.C. and Young, S.Y. (1977). Inactivation of Baculovirus heliothis by ultraviolet irradiation, dew and temperature. J. Invertebr. Pathol. 30: 237-241.

Morris, O.N. (1962a). Studies on the causative agent and histopathology of a virus disease of the western Oak looper. J. Insect Pathol. 4: 446-453.

Morris, O.N. (1970). Precocious development of adult characteristics in virus infected Lepidoptera. J. Invertebr. Pathol. 16: 173-179.

Morris, O.N. (1971). The effect of sunlight, ultraviolet and gamma radiation and temperature on the infectivity of a nuclear polyhedrosis virus. J. Invertebr. Pathol. 18: 292-294.

Morris, O.N. and Moore, A. (1975). Studies on the protection of Insect pathogens from sunlight inactivation. 11. Preliminary field trials. Chemical control of Research Institute, Ottawa, Ontario, Report cc-x-113.

Nair, K.P.V. (1981). Investigations on the nuclear polyhedrosis of rice swarming caterpillar, Spodoptera mauritia (Boisduval) Ph.D. Thesis, Kerala Agricultural University, Vellanikkara, Trichur.

Nair, K.P.V. and Jacob, A. (1976). Studies on the nuclear polyhedrosis of Pericallia ricini F. (Lepidoptera: Arctiidae) Entomon 1: 23-30.

Nair, K.P.V. and Jacob, A. (1985). Transmission of nuclear polyhedrosis virus of rice swarming caterpillar. Spodoptera mauritia (Boisduval) through egg. Entomon 10(1) : 1-6.

Narayanan, K. (1979). Studies on nuclear polyhedrosis virus of gram caterpillar Heliothis armigera(Hb). (Noctuidae: Lepidoptera). Ph.D. thesis. Tamil Nadu Agricultural University, Coimbatore.

Narayanan, K. (1980). Effect of NPV of Heliothis armigera (Hubn.) and Spodoptera litura (F.) on parasite, predators and silkworm. Proc. of the third workshop of All India Coordinated Research Project on Biological control of crop pests and weeds. Punjab Agril. Univ. Ludhiana. Oct. 1980. 27-30.

Narayanan, K., Govindarajan, R. and Jayaraj, S. (1977a). Preliminary observations on the persistence of nuclear polyhedrosis virus of Spodoptera litura F. Madras Agric. J. 64: 687-688.

Narayanan, K., Easwaramoorthy, S., Santharam, G., Jayaraj,S. and Muthu, M. (1977b). Exposure of white mice to Baculovirus gmscata to groundnut red hairy caterpillar. Amscata albistriga Wlk. Curr. Sci. 46(12): 417-419.

Narayanan, K., Govindarajan, R., Jayaraj, S., Raj, S.P. and Kutty, M.N. (1977c). Non-susceptibility of common carp. Cyprinus carpio L. to nuclear polyhedrosis virus, Baculovirus amscata of groundnut hairy caterpillar. Madras Agric. J. 64(6): 411-412.

Narayanan, K., Santharam, G., Easwaramoorthy, S. and Jayaraj, S. (1978). Lack of susceptibility of poultry birds to nuclear polyhedrosis virus of groundnut Red hairy caterpillar. Amscata albistriga(W). Indian J. of Exp. Biol. 16 (2): 1322-1324.

Neelagund, Y.F. and Mathad, S.B. (1978). Transmission of nuclear polyhedrosis virus in laboratory population of army worm, Mythimna (Pseudaletia) separata. J. Invertebr. Pathol. 31: 143-147.

Nordin, G.L. (1976). Transovum transmission of a nuclear polyhedrosis virus of the fall webworm Hyphantria cunea (Lepidoptera: Arctiidae) J. Kans. Entomol. Soc. 49: 589-594.

Nordin, G.L. (1977). Inactivation of a baculovirus of Hyphantria cunea by sunlight and ultraviolet irradiation (Lepiodoptera: Arctiidae). J. Kans. Entomol. Soc. 50(1): 18-22.

Oatman, E.R., Hall, I.M., Arakawa, K.Y., Planter, G.R., Bascom, L.A. and Beegle, C.D. (1970). Control of the corn Earworm on Sweet corn in Southern California with a nuclear polyhedrosis virus and Bacillus thuringiensis. J. econ. Ent. 63: 415-421.

Odinoo, M.O. (1981).Dosage-mortality and time mortality response of the armyworm Spodoptera exempta to a nuclear polyhedrosis virus. J. Invertebr. Pathol. 38(2): 251-255.

Okada, T. (1970). A histopathological study on the nuclear polyhedrosis of larvae of Spodoptera litura F. (Lepidoptera: Noctuidae). Proc. Assoc. Pl.Prot. Kyushu. 16: 40-41.

Okada, T. (1972). Persistence of a nuclear polyhedrosis virus of Spodoptera litura Fabricius mixed with gelatin solution. Proc. Assoc.Pl.Prot. Kyushu. 18: 15-17.

Okada, T. (1974). Persistence of viral activity of Spodoptera litura nuclear polyhedra sprayed in mixtures. Proc. Assoc. Pl.Prot. Kyushu. 20: 37-39.

Ossowski, L.L.G. (1960). The biological control of the wattle bagworm Kotochalia junodi (Heyl.) by a virus disease. II. Large scale experiment. Ann. appl. Biol. 48: 299-313.

Patel, R.C., Singh, R. and Patel, P.B. (1968). Nuclear polyhedrosis of the gram pod borer, Heliothis armigera. J. econ. Ent. 61: 191-193.

Pawar, V.M. (1975). Structure, development and Physico-chemical properties of the nuclear polyhedrosis virus of Spodoptera litura (Fabricius) and associated biochemical changes in the haemolymph of the host. Entomologist's News letter 5: 14-15.

Pawar, V.M. and Ramakrishnan N. (1971). Investigations on the nuclear polyhedrosis of Prodenia litura F. Nature of the polyhedral diseases. Indian J. Ent. 33: 111-122.

Pawar, V.M. and Ramakrishnan, N. (1974). Investigations on the nuclear polyhedrosis of Prodenia litura Fab.I. Nature of the polyhedral diseases. Indian J. Ent. 33(2): 111-122.

Pawar, V.M. and Ramakrishnan, N. (1975). Investigations on the nuclear polyhedrosis of Spodoptera (Prodenia) litura (Fabricius) III. Bioassay of viral activity. Indian J. Ent. 37: 367-374.

Pawar, V.M. and Ramakrishnan, N. (1977). Investigations on the nuclear polyhedrosis of Spodoptera litura Bioassay of virus activity. Indian J. Ent. 37(4): 367-374.

Pawar, V.M. and Ramakrishnan, N. (1979). Stability of Baculovirus of Spodoptera litura (Fabricius). J. Ent. Res. 1: 206-212.

Peter, Z. (1981). A nuclear polyhedrosis virus (Baculovirus-sub group A) isolated from Polia dissimilis Knoch. (Lepidoptera: Noctuidae) Analele Institutului de Cercetari pentru Protectia Plantelor. 16: 233-236.

Philip, B.M., Mathai, S. and Jacob, A. (1982). A nuclear polyhedrosis virus of black headed caterpillar. Nephantis serinopa (Cryptophasidae: Lepidoptera). Curr. Sci. 51(12): 611.

Pillai, G.B. and Nair, K.R. (1982). A method to estimate the intensity of natural pupal parasitism of Opisina arenosella Wlk. (= Nephantis serinopa Meyrick). J. Plant. Crops. 10(2): 86-91.

Ponson, M.B. and Dejong, D.J. (1964). A nuclear polyhedrosis of Orthesia incerta (Hufnagel)(Lepidoptera: Noctuidae). J. Insect Pathol. 6: 376-378.

Rabindra, R.J. and Subramaniam, T.R. (1974). Studies on the nuclear polyhedrosis of Heliothis armigera. Madras Agric. J. 61: (10/12): 957-961.

Rabindra, R.J. and Subramaniam, T.R. (1975a). Histopathology of Dasychira mendosa Hb. (Lepidoptera: Lymantriidae) infected with polyhedrosis virus. Curr. Sci. 44: 591-592.

Rabindra, R.J. and Subramaniam, T.R. (1975b). Nuclear polyhedrosis of Plusia peponis F. (Lepidoptera: Noctuidae) Curr. Sci. 44: 634

Rabindra, R.J. and Subramaniam, T.R. (1976). Nuclear polyhedrosis of Plusia chalcytes. Esp. infective to Plusia peponis F. Curr. Sci. 45: 564-565.

Rabindra R.J., Paul, A.V.N., David, B.V. and Subramaniam, T.R. (1975). On the nuclear polyhedrosis of Pulsia chalcytes Esp. (Lepidoptera: Noctuidae). Curr. Sci. 44: 273-274.

Raimo, B., Reardon, R.C. and Podgwaite, J.D. (1977). Vectoring gypsy moth nuclear polyhedrosis virus by Apanteles melanoscaules (Hym. Braconidae) Entomophaga 22(2): 207-215.

Ramakrishnan, N. and Tiwari, L.D.(1969). Polyhedrosis of Prodenia litura Fabr. (Noctuidae: Lepidoptera) Indian J. Ent. 31: 191-192.

Ramosk, W.A., Stairs, G.R. and Hink, W.F. (1975). Ultraviolet activation of insect nuclear polyhedrosis virus. Nature Lond. 253: 628-629.

Rao, A.S. and Amonakar, S.U. (1980). Nuclear polyhedrosis of Amscata moorei. Butler, (Lepidoptera: Arctiidae) Curr. Sci. 49(19): 709-710.

Rollinson, W.D., Lewis, F.B. and Waters, W.E. (1965). The successful use of a nuclear polyhedrosis virus against the gypsy moth. J. Invertebr. Pathol. 7 515-517.

Roome, R.E. and Daoust, R.A. (1976). Survival of the nuclear polyhedrosis virus of Heliothis armigera on crops and in soil in Botswana. J. Invertebr. Pathol. 27: 7-12.

Sager, S.M. (1960). On the transtadial transmission of insect viruses. J. Insect Pathol. 2: 307-309.

Santharam, G. and Balasubramonian, M. (1980). Note on the control of Spodoptera litura Fabricius (Lepidoptera: Noctuidae) on tobacco with a nuclear polyhedrosis virus and diflubenzuron. Indian J. agric. Sci. 50: 726-727.

Santharam, G., Regupathy, A., Easwaramoorthy, S. and Jayaraj, S. (1980). Effectiveness of nuclear polyhedrosis virus against field population of Spodoptera litura Fab. on banana. Indian J. agric. Sci. 48(11): 676-678.

Sidor, C. (1960). A Polyhedral disease of Chrysopa perla L. Virology. 10: 551.

Smirnoff, W.A. (1961). A virus disease of Neodiprion swaini Middleton. J. Insect Pathol. 3: 29-46.

Smirnoff, W.A. (1962). Transovum transmission of insect viruses as a means of biological control. Coll. Int. Pathol. Insects Paris No.2. pp.459-460.

Smirnoff, W.A. (1976). Susceptibility of Hyphantria cunea (Lepidoptera: Noctuidae) to infection by NPV of Thymelicus lineola (Lepidoptera: Hesperiidae). Can. Ent. 108: 327-330.

Smirnoff, W.A. and Ackermann, H.W. (1977). A baculovirus of the European skipper, Thymelicus lineola (Lepidoptera: Hesperiidae) J. Invertebr. Pathol. 30 (2): 224-231.

Smith, K.M. (1967). Insect virology. Academic Press, New York. 256 pp.

Smith, K.M. and Xeros, N. (1954). An unusual virus disease of a dipterous larvae. Nature Lond. 173: 366-367.

Smith, K.M., Hill, G.J. and Rivers, C.G. (1959). Polyhedrosis of neuropterous insects. J. Insect Pathol. 1: 431-437.

Stairs, G.R. (1965a). The effect of metamorphosis on nuclear polyhedrosis virus infection in certain Lepidoptera. Can J. Microbiol. 11: 509-512.

Stairs, G.R. (1965b). Quantitative difference in susceptibility to nuclear polyhedrosis virus among larval instars of forest tent caterpillar. Malacosoma distria. J. Invertebr. Pathol. 7: 427-430.

Stairs, G.R. (1978). Effect of wider range temperatures on the development of Galleria mellonella and its specific baculovirus. Environ. Entomol 7(2): 297-299.

Stairs, G.R. and Bird, F.T. (1962). Dissemination of viruses against spruce budworm, Choristoneura fumiferana (Clemens). Can. Ent. 94: 966-969.

Stairs, G.R. and Milligan, J.E. (1980). Quantitative effect of heat on baculovirus virions released from polyhedra (Nuclear polyhedrosis virus- Galleria mellonella) Environ. Entomol. 9(5): 586-588.

Stairs, G.R., Fraser, T. and Fraser, M. (1981). Changes in growth and virulence of a nuclear polyhedrosis virus from Choristoneura fumiferana after passage in Trichoplusia ni and Galleria mellonella. J. Invertebr. Pathol. 38(2): 230-235.

Steinhaus, E.A. (1949a). "Principles of Insect Pathology". 757 pp. McGraw Hill book co., New York.

Sugun, S., Mathad, S.B. and Reddy, S.J. (1983). Histopathology of the armyworm, Mythimna (Pseudaletia) separata Wlk. (Lepidoptora: Noctuidae) infected with nuclear polyhedrosis virus. Entomon 8(2): 155-162.

Summers, M.D., Engler, R., Falcon, L. and Vail, A. (eds) (1975). Baculovirus for Insect pest control: Safety considerations. Am. Soc. Microbiol. Washington. 181 pp.

Swaine, G. (1966). Generation to generation passage of nuclear polyhedral virus of Spodoptera exemota (Wlk) Nature. Lond. 210: 1053-1054.

Tanada, Y. (1954). A polyhedrosis virus of the imported cabbage worm and its relation to a polyhedrosis virus of alfalfa caterpillar. Ann. ent. Soc. Am. 47: 553-574.

Tanada, Y. (1956). Some factors affecting the susceptibility of the armyworm to virus infections. J. econ. Ent. 49: 52-57.

Tanada, Y. (1959). Descriptions and characteristics of a nuclear polyhedrosis virus and a granulosis virus of the armyworm, Pseudaletia unipuncta (Haworth) (Lepidoptera: Noctuidae) V. Insect Pathol. 1: 197-214.

Tanada, Y. (1960). A nuclear polyhedrosis of the lawn armyworm Spodoptera mauritia (Boisduval) in Hawaii (Lepidoptera: Phalaenidae) Proc. Hawaiian Ent. Soc. 16: 411-436.

Tanada, Y. and Hess, R.T. (1970). Development of a nuclear polyhedrosis virus in midgut cells and penetration of the virus into haemocoel of the armyworm Pseudaletia unipuncta. J. Invertebr. Pathol. 23: 360-365.

Tanada, Y and Kelner, C. (1962). The use of pathogen in the control of the corn ear worm, Heliothis zea (Boddie) J. Insect Pathol. 4: 139-154.

Thompson, J.G. (1959). Thermal inhibition of certain polyhedrosis virus disease. J. Insect Pathol 1: 189-190.

Timans, U. (1982). The effect of ultraviolet radiation on the nuclear polyhedrosis virus of the gypsy moth, Lymantria dispar L. (Lep: Lymantriidae). Z. angew Ent. 94(4): 382-401.

Tinsely, T.W. (1975). Viruses as pesticidal agents: potential and limitations. Proc. 8th Br. Insectic Fungic. Cont Bringhton. 1975.

Tinsely, T.W. (1979). The potential of insect pathogenic viruses as pesticidal agents. Ann. Rev. Entomol. 24: 63-67.

Turner, G.. and Kaplan, C. (1965). Observations on photo-dynamic inactivation of vaccinia virus and its effect on immunogenicity. J. Hyg. Camb. 63: 395.

Vail, P.V. and Gough, D. (1970). Susceptibility of the pupal stage of the cabbage looper, Trichoplusia ni, to nuclear polyhedrosis virus II. Histopathology. J.Invertebr. Pathol. 15: 211-218.

Vail, P.V. and Hall, I.M. (1969a). The histopathology of a nuclear polyhedrosis in larvae of the cabbage looper, Trichoplusia ni related to symptoms and mortality. J. Invertebr. Pathol. 13: 188-198.

Vail, P.V. and Hall, I.M. (1969b). The influence of infection of nuclear polyhedrosis virus on adult cabbage looper and their progeny. J. Invertebr. Pathol. 13: 358-370.

Vail, P.V., Jay, D.L. and Hunter, D.K. (1972). Cross infectivity of a nuclear polyhedrosis virus isolated from the alfalfe looper, Autographa californica. Ent. Res. Division, ARS 1972. pp.297.304.

Vail, P.V., Seay, R.T. and DeBolt, J. (1980). Microbial and chemical control of cabbage looper on Fall lettuce. J.Econ. Ent. 73: 72-73.

Watanabe, S. (1951). Studies on the grassorie virus of the silkworm Bombyx mori: Physical and chemical effects on the virus. Jap. J.Exp. Med. 21: 299-313.

Watanabe, S. (1968). Abnormal cell proliferation in the epidermis of the fall webworm, Hyphantria cunea induced by the infection of a nuclear polyhedrosis virus. J. Invertebr. Pathol. 12: 310-315.

Wittig, G. (1963). Techniques in Insect Pathology. In "Insect Pathology an advanced treatise" (E.A. Steinhaus, ed.) Vol.2, pp.591-636. Academic Press, New York.

Witt, D.J. and Stairs, G.R. (1977). The effect of UV irradiation on a baculovirus infecting Galleria mellonella. J.Invertebr. Pathol. 26: 321-327.

Xeros, N. (1956). The Virogenic stroma in nuclear and cytoplasmic polyhedroses. Nature Lond. 173: 412-413.

Young, S.Y. and Yearian, W.C.C. (1974). Persistence of Heliothis NPV on foliage of cotton, soybean and tomato. Environ. Entomol. 3: 253-255.

* original not seen.

APPENDICES

Appendix I

Abstract of ANOVA

		df	Mean squares
Table 8	Total	23	
	Treatment	5	4.9673**
	Block	3	0.0455
	Error	15	0.0565
Table 9	Total	44	
	Treatment	14	1012.51*
	Error	30	185.73
Table 10	Total	41	
	Treatment	13	1378.84**
	Error	28	35.95
Table 11	Total	41	
	Treatment	13	1779.47**
	Error	28	71.73

* Significant at 5% level

** Significant at 1% level

Appendix II

Response of O prengoselia larva to different doses of Nuclear polyhedrosis virus administered to different instars (Vide Table No 6)

	Dose	Cumulative per cent mortality observed at intervals (in days)													
		2	3	4	5	6	7	8	9	10	11	12	13	14	15
	First instar														
1	1 96 x 10^5 PIBs/ml	16	48	74	100										
2	1 96 x 10^4 PIBs/ml	14	34	58	94	100		-		-					
3	1 96 x 10^3 PIBs/ml	22	30	42	68	92									
4	1 96 x 10^2 PIBs/ml	0	0	12	24	32	38	52	78		—				
5	1 96 x 10 PIBs/ml			17 5	25	35	40	47 5	52						
6	1 96 x				10	17	25	30	35	38		-	-		
	Second instar														
1	1 96 x 10^7 PIBs/ml		20	36	58	80	100	-	-	-	-				
2	1 96 x 10^6 PIBs/ml		14	34	64	80	100	-	-		-	-			
3	1 96 x 10^5 PIBs/ml	-	10	36	56	78	96	-	-	-					
4	1 96 x 10^4 PIBs/ml	-	10	32	50	64	84								
5	1 96 x 10^3 PIBs/ml	-	4	6	14	26	52	76	-	-	-				
6	1 96 x 10^2 PIBs/ml		6	8	8	18	28	48							
	Third instar														
1	1 96 x 10^7 PIBs/ml		26	36	50	74	100			-	-	-			
2	1 96 x 10^6 PIBs/ml		14	34	58	80	96		-						
3	1 96 x 10^5 PIBs/ml		8	8	16	26	48	80							
4	1 96 x 10^4 PIBs/ml	4	4	6	14	40	72	-							
5	1 96 x 10^3 PIBs/ml		-	4	6	8	12	30	52						
6	1 96 x 10^2 PIBs/ml				4	6	10	16	24	40					
	Fourth instar														
1	1 96 x 10^7 PIBs/ml		6	12	30	54	72	86							
2	1 96 x 10^6 PIBs/ml		6	18	32	50	66	74	84	-					
3	1 96 x 10^5 PIBs/ml		6	10	20	36	50	66	70	76					
4	1 96 x 10^4 PIBs/ml	-	4	6	20	24	36	46	46	62					
5	1 96 x 10^3 PIBs/ml			4	6	10	10	14	18	28	46				
6	1 96 x 10^2 PIBs/ml					2	2	8	12	14	20	38			
	Fifth instar														
1	1 96 x 10^7 PIBs/ml		2	4	4	6	8	28	60	62					
2	1 96 x 10^6 PIBs/ml		2	2	2	4	8	30	42	56	-				
3	1 96 x 10^5 PIBs/ml			2	2	2	6	6	20	36	52				
4	1 96 x 10^4 PIBs/ml			2	2	2	4	6	18	30	4.				
5	1 96 x 10^5 PIBs/ml							2	2	4	6	16	30		
6	1 96 x 10^2 PIBs/ml	-					-	2	2	2	2	6	6	14	

Appendix III

Abstract of Analysis of variance table relating to Table 17a to c

	df	MS (Area I) (a)	MS (Area II) (b)	MS (Area III) (c)
Period	14	0.8255*	9.3396**	7.5499**
Treatment	2	6.7852**	9.1558**	12.7861**
P x T	28	0.3381**	2.8836**	1.7906**
Error (Pooled)	405	0.0115	0.0138	0.0099

* Significant at 5% level

** Significant at 1% level

Appendix IV

Abstract of pooled ANOVA (relating to Table 18a and b)

Source	df	Mean squares	
		Table 18a	Table 18b
Period	4	83.331	215.709
Treatment	2	2.463	1.833
P x T	8	9.813	18.891
Error (Pooled)	20	7.637	19.36

NUCLEAR POLYHEDROSIS OF *Opisina arenosella* WLK. (= *Nephantis serinopa* MEYR:) (CRYPTOPHASIDAE: LEPIDOPTERA) AND ITS UTILITY FOR THE CONTROL OF THE PEST

BY

BABU M. PHILIP

ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENT FOR THE DEGREE

DOCTOR OF PHILOSOPHY

KERALA AGRICULTURAL UNIVERSITY

FACULTY OF AGRICULTURE

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, TRIVANDRUM

1985

ABSTRACT

The black headed caterpillar, Opisina arenosella Wlk. (= Nephantis serinopa Meyrick) is a serious pest of coconut. The slender caterpillars found under the leaves in galleries made of silk and foreign matter feed on the chlorophyll tissues. Philip et al., (1982) reported the occurrence of a nuclear polyhedrosis virus in the larvae of O. arenosella. Basic information for judging the suitability of this pathogen for the control of the pest were lacking. Hence detailed studies were made on the symptomatology, morphology of the pathogen, histopathology, mode of transmission of the virus to the progeny, cross-infectivity to other species of lepidoptera, bioassay of the virus, persistence of the virus on coconut foliage, effect of physical factors on the persistence of the virus, safety of the pathogen to non-target organisms and the efficacy of the virus in controlling the pest in field.

The required life stages of the pest for various experiments were obtained from laboratory cultures maintained under aseptic condition.

The symptoms of the disease resembled those reported already for the nuclear polyhedroses of other lepidopteran larvae. The typical behaviour of coming to the open and

orienting the body with the head hung downwards at the time of death, was not observed in the case of O. arenosella. Some of the diseased pupae exhibited typical symptom of larval pupal mosaic. Malformed adults with short and ruffled wings also emerged.

The inclusion bodies measured on an average 1393.30 nm with a range of 533.33 nm to 1666.67 nm. The inclusion bodies were irregular in shape and the surface was smooth without any ornamentation. The pathogen was found to be a multiple embedded virus.

The histopathological studies revealed that the hypodermis, fat body and trachea were the principal sites of virus multiplication. In addition to the above tissues, muscles, Malpighian tubules, connective tissues surrounding the gut, the gut epithelium, nerve ganglia and epithelial sheath of gonads were seen infected. The extensive invasion of the tissues indicated the high virulence of the pathogen.

The female moths of O. arenosella infected orally or contaminated externally served as effective carriers of the pathogen to the next generation mainly through surface contamination of eggs (transovum). This facilitated the natural perpetuation and spread of the disease.

The NPV of *O. arenosella* was found cross transmissible to *S. litura*, *S. mauritia*, *A. peronis*, *P. ricini* and *E. obliqua*. *S. litura* and *S. mauritia* were found suitable alternate hosts for large scale multiplication of the virus by virtue of their amenability to laboratory rearing. The alternate hosts themselves being major crop pests, the possibility of using the virus as a broad spectrum microbial insecticide was also indicated.

Bioassay studies revealed the desirability of applying the virus in the early stages of pest incidence since the later instars required very heavy doses of the pathogen and that might render the technology non-viable.

The NPV of *O. arenosella* when exposed to field condition (weathering) retained substantial infectivity upto 84 hours and then there was a decline in virulence causing complete inactivation in 168 hours. The half life of the pathogen on coconut foliage was 83.25 hours. This compared well with many of the insecticides.

When exposed to a constant temperature of 35°C the infectivity declined rapidly beyond 84 hours and complete inactivation occurred at 156 hours of exposure. The half life of the virus was 87.10 hours. When the NPV of

O. arenosella was exposed to infrared rays, substantial infectivity was retained upto 84 hours. The half life of the virus was 75.34 hours. Exposure to UV rays caused rapid loss of infectivity resulting in complete inactivation in 108 hours. The half life of the virus was 54.43 hours. The most important factor inactivating the virus was thus found to be the UV rays in sunlight.

The virus was found to be comparatively safe to silkworm (Bombyx mori), parasitoids (Bracon brevicornis and Trichospilus pupivora), Vertebrates (white rats and white mice) and even to the embryonated chick eggs.

In the field evaluation of NPV against O.arenosella the virus spray containing 22.14 x 10^7 PIBs/ml came on par with the insecticidal treatment of thiodan 0.05 per cent.

The present studies revealed the high virulence of the NPV, its ability to spread from parent to progeny, a reasonable persistence on the treated foliage and safety to non-target organisms. It is a promising microbial pesticide for the control of O. arenosella. The NPV can fit well in an integrated control programme against O. arenosella.