STUDIES ON NUCLEAR POLYHEDROSES OF THREE SPECIES OF LEPIDOPTERA

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INTRODUCTION

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Problems such as insect resistance, hazards and environmental pollution inherent in the use of chemical insecticides have made it necessary to seek non-insecticidal materials and methods to combat the insect foes. Microbial control making use of pathogenic microorganisms is one among the important non-insecticidal control measures. There has been a great increase in our knowledge of microbial diseases of insects during recent years. The principal groups of organisms responsible for infectious disease in insects are: viruses, bacteria, fungi, protozoa, nematodes and rickettsiae.

The diseases caused by viruses are perhaps the most spectacular of all afflictions to which these arthropods are subject. According to Ignoffo (1968) virus diseases represent approximately 25 per cent of the total recorded diseases of insects and at a minimal estimate 12 to 15 new viruses are described each year. The field of insect virology has broadened considerably during recent years and much headway has been made in this line in countries like U.S.A., Canada, U.K., France and the U.S.S.R. There are several cases of successful

use of insect viruses, especially the nuclear polyhedroses and granuloses, in the control of insect pests (Balch and Bird, 1944; Thompson and Steinhaus, 1950; Bird, 1953; Hall, 1957; Kelsey, 1957; McEwen and Harvey, 1958; Abul-Nasr, 1959; Ossowski, 1959; Tanada and Reiner, 1962; Ignoffo et al., 1965).

Studies on insect viruses are, however, rather limited in India. Only very few virus diseases of insect pests have been reported so far in India and these include a nuclear polyhedrosis of Heliothis armigera (Patel et al., 1968) and Prodenia litura (Ramakrishnan and Tiwari, 1969) and a pox-like virus disease of Amsacta moorei (Roberts and Granados, 1968; Mathur, 1971). These studies are only preliminary in nature and no detailed information on the host-pathogen relationships is available on any of these diseases.

In view of the above, the present studies were taken up. The study included a preliminary survey of important lepidopterous crop pests of Coimbatore, Tamil Nadu, for the presence of nuclear polyhedrosis. Some general observations were made on the diseases detected with a detailed study on one of them viz., the nuclear polyhedrosis of Spodoptera litura F. covering the symptomatology, histopathology and some of the biochemical changes in the larval body.

REVIEW OF LITERATURE

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Insect viruses and the diseases caused by them have been the subjects of several authoritative reviews in recent years (Bergold, 1953, 1958, 1963; Smith, 1955, 1959 a, 1962, 1963, 1967; Aizawa, 1963; Aruga, 1963; Huger, 1963; Ignoffo, 1968; Vago and Bergoin, 1968). Pathophysiology of virus infected insects has been reviewed by Bergold (1959), Aizawa (1963), Benz (1963 a) and Martignoni (1964 a). The literature cites over 30 examples from different parts of the world in which arthropod viruses were used to control economically important pests (Ignoffo, 1968). These examples are cited in the reviews of Smith and Williams (1958), Tanada (1959 a), Franz (1961), Cameron (1963), Vago (1964), Heimpel (1965) and Ignoffo (1966, 1967, 1968). The mass production of viral pathogens has been dealt with by Martignoni (1964 b), Vasiljevic (1964) and Ignoffo (1966). Hence a detailed review is not attempted here. Literature relevent to the present investigation alone is reviewed hereunder.

Nuclear polyhedrosis of *Heliothis* spp.

Mally (1891, 1892) working in North America, quoted by Steinhaus (1949 a), first reported a wilt

disease in the larvae of H.armigera. Lounsbury (1913) made a fragmentary reference to a "caterpillar wilt" of H.obsectus from lucerne fields of South Africa. Chapman and Glaser (1915) listed the corn earworm, H.obsoleta F. (= H.zea (Boddie)) among 34 species of insects showing wilt diseases. Parsons (1936) was the first to definitely incriminate a virus as the causative agent of wilt disease of the boll worms, H.armigera and he could artificially transmit the disease in the laboratory. Stahler (1939) observed that the diseased larvae of H.armigera assumed a metallic lustre, stopped feeding, became paralyzed and failed to moult. Upon death the cuticle blackened and became soft and sticky so that the least tension pulled it apart allowing the liquefied body contents to flow out. When the larvae were fed on lettuce or alfalfa the mortality was higher than when they were fed on tomato fruits or corn meal. No polyhedral bodies were detected. The first photomicrographs of inclusion bodies and partially occluded virions of H.armigera nuclear polyhedrosis were published by Smith and Rivers (1956). Additional photomicrographs of virions from H.armigera and H.zea were later published by Bergold and Ripper (1957) and Gregory et al. (1969) respectively.

Steinhaus (1949 a) reported a wilt disease of the larvae of *A.phloxiphaga* and observed the presence of polyhedral bodies in the infected larvae. He (1957) found a nuclear polyhedrosis infection in larvae of the tobacco budworm, *H.virescens* collected from Louisiana. Coaker (1955) reported the occurrence of a polyhedral disease in the larvae of *A.armigera* on cotton plants in Uganda and he (1958) described the symptoms of the disease. The diseased larvae developed typical wilt symptoms and they were usually found suspended from the food plant by the hind prolegs as a limp and flaccid sac filled with liquefied body contents. The body wall readily ruptured on touch. The diseased larvae were also found as blackish stains on the foliage where they had burst liberating the liquefied body contents.

A nuclear polyhedrosis of the safflower leaf worm *H.peltigera* (Schiff.) was reported from Israel by Harpaz and Zlotkin (1965). The symptoms and pathology of the disease generally resembled those described for other nuclear polyhedroses of lepidopterous larvae. However, the hanging down of cadavers, commonly regarded as distinctive of death by nuclear polyhedrosis was found to be a secondary and non-characteristic manifestation. Histopathological studies showed

infection of the fat body, hypodermis, tracheal matrix, blood cells and midgut epithelium.

In a study of the virulence of nuclear polyhedrosis virus of H.zea and H.virescens, Ignoffo (1965) observed that initial larval mortality occurred 3 days after inoculation and approximately 95 per cent of the total mortality was reached on the sixth day for H.zea and on seventh day for H.virescens. Allen and Ignoffo (1969) reported that based on LD_{50} values, 3 days old larvae of H.zea were more susceptible than 5 days old ones to a nuclear polyhedrosis virus.

According to Bergold and Ripper (1957) the polyhedra isolated from diseased larvae of H.armigera were irregular in shape and varied in diameter from 0.7 to 1.2 μ, the majority measuring 1.1 μ. The virus particles were rod shaped and arranged singly, though there were very few virus bundles containing most likely only two rods. The average dimensions of the single virus rods were about 320 $\pm$ 10 mμ x 90 $\pm$ 10 mμ. These authors named the virus they isolated as Borrelina armigera. Cooker (1958) observed the polyhedra from H.armigera to vary from 1 to 2 μ in diameter. The polyhedra isolated from H.peltigera

varied from 1 to 3 μ in diameter (Harpaz and Zlotkin, 1965). Gregory et al. (1969) described the inclusion bodies and virions of H.zea nuclear polyhedrosis. The polyhedra were irregular and apparently six sided in outline. The average diameter was 916.4 ± 9.5 mμ with a range of 300 to 2240 mμ. No surface patterns or prominent structures were apparent. The virions occurred singly and bundles of virions were never observed. The virus rods measured 336 ± 22 x 62 ± 4 mμ on an average.

In India, Patel et al. (1968) reported the incidence of a nuclear polyhedrosis in a laboratory culture of H.armigera in Gujarat giving a brief note on the symptoms.

Nuclear polyhedrosis of Spodoptera spp.

Crumb (1929) reported the occurrence of a disease presumed to be polyhedrosis, in Prodenia litosia in Europe. According to Blanchard and Conger (1932) a polyhedral disease was the most important factor in the natural control of the yellow striped army worm, P.praefica Grote in central and northern California, western Nevada and southern Oregon. This disease was reported to affect the larvae in the fourth and last

instars turning them reddish brown before death after which the body cells disintegrated rapidly into a dark watery mass which hung limply on the leaves and stems. Steinhaus (1949 a) published the photomicrographs of the virus rods of the nuclear polyhedrosis of P.praefica and later described and named it as Borrelina olethria (Steinhaus, 1949 b, 1953). The occurrence of this disease in Egypt was recorded by Abul-Nasr (1954) and in different parts of California by Steinhaus and Marsh (1962). According to Steinhaus (1960) the affected larvae turned reddish brown before death. The lateral stripes and dorsum of the young larvae became pinkish in colour. There appeared to be an accumulation of pink granules in the clear areas of the integument. Infected larvae swelled up considerably.

Laphygma frugiperda (= Spodoptera frugiperda) was listed by Chapman and Glaser (1915) as being susceptible to a polyhedrosis virus and Allen (1921) reported an outbreak of this disease in Mississipi. Later Steinhaus and Marsh (1962) noted its occurrence in Louisiana. A nuclear polyhedrosis of Laphygma exigua (= S.exigua) was first reported by Steinhaus (1949 b, 1951) who photographed the virus rods. The

virus rods measured approximately 270 x 40 mμ. According to Hunder and Hall (1968) the polyhedra measured 2.05 μ on an average with a range of 1 to 6 μ and the virus rods always occurred in bundles of 2 to 7 rods.

The occurrence of a nuclear polyhedrosis in P.ornithogalli (Guenee) was first reported by Steinhaus (1949 a). Later in 1962, Steinhaus and Marsh noted its occurrence in various parts of California. Diseased specimens of another armyworm, P.terricola (F.) received from Ceylon was diagnosed by Steinhaus and Marsh (1962) as affected by a nuclear polyhedrosis. The virus rods measured approximately 300 x 60 mμ.

A nuclear polyhedrosis of the lawn armyworm, Spodoptera mauritia (Boisduval) was discovered in Hawaii by Bianchi (1957). According to Tanada and Beardsley (1958) this virus disease played an important role in the natural control of the lawn armyworm in Hawaii. Tanada (1960) described the symptoms and causative agent of this disease. The larvae shortly after succumbing to the virus showed the typical symptoms of nuclear polyhedrosis. Larvae in the early instars turned pale two to four days before death and at death had a whitish or creamy appearance. Older larvae when

infected gradually turned slightly pale with a pinkish tinge several days before death, though the change in colouration was not marked as in the younger larvae. The period of lethal infection varied with instars from 3 to 10 days. The first six larval instars were highly susceptible to the virus, but the last instar seemed to be fairly resistant. The diameter of polyhedra varied from 1.07 to 3.22 μ with a mean of 1.61 μ. The virus rods measured 324.7 + 3.37 x 61.9 + 0.59 mμ.

A disease, probably the nuclear polyhedrosis, of P.litura was reported as early as 1913 in Egypt, where there was a heavy mortality of the insect as a result of infection (Dudgeon, 1913). This insect had been similarly attacked in Indo China where Caresche (1937) transmitted the disease to healthy larvae by feeding them on leaves treated with an extract from diseased individuals. Later in 1937, Wilcox and Bhaget also observed this disease in Egypt. Abul-Nasr (1954, 1956) found that this disease was a typical nuclear polyhedrosis. The natural infection in the field ranged from 5 to 20 per cent in different seasons. The disease developed rapidly in summer and slowly in winter, the larvae dying in 4 to 5 days in summer and

full symptoms being developed in 10 to 12 days in winter. Bergold and Flaschentrager (1957) isolated the polyhedra and virions from diseased larvae collected from Egypt and they named the virus as Borreline litura. The polyhedra were irregular in shape and varied in diameter from about 1.2 to 3.2 μ, the majority measuring about 1.8 μ. The virus rods occurred in bundles of several particles and there were comparatively few single virus particles. The average dimensions of single rod still possessing developmental membrane were about 320 $\pm$ 20 x 100 $\pm$ 10 mμ.

The polyhedral viral disease of P.litura in Egypt was further investigated by Hafez (1958). When first, third and fourth instar larvae kept in batches of 100 in laboratory cages, were fed on clover leaves sprayed in the field with a suspension of 25 million polyhedra/ml, all died within 11 days. Loss of appetite appeared in 3 to 4 days before death. When full grown larvae were infected artificially there was 59 to 73 per cent mortality among the resultant pupae as compared with 33 to 36 per cent among control and adults emerging from the survivors were malformed, did not oviposit and soon died. Field tests conducted by Abul-Nasr (1959) on cotton, sweet potato and maize showed that the pest

population was reduced below the economic level in cotton and sweet potato. Grown up *Prodenia* larvae on maize were not successfully infected.

Studies by Harpaz and Ben-Shaked (1964) indicated that the generation to generation transmission of the nuclear polyhedrosis of *P.litura* involved a rather complicated genetic mechanism. Further experiments by Ben-Shaked and Harpaz (1966) indicated that the endocrine mechanism regulating growth and development was involved in the activation of latent nuclear polyhedrosis in *S.littoralis*. Studies by Kislev *et al.* (1971) on the characterisation of nuclear polyhedrosis viral DNA and its comparison to the host DNA suggested a latent infection of *S.littoralis*. Kislev *et al.* (1969) reported that the polyhedra of *S.littoralis* measured on an average 1.8 μ in diameter with a range of 1 to 3 μ. They further observed that out of the four major types of haemocytes differentiated in the blood of this insect, virus formation occurred mainly in the plasmatocytoids and only to a much lesser extent in the granular haemocytes and oenocytoids. Adipohaemocytes were never seen to sustain virus development. Plasmatocytoids were observed phagocytosing free virus particles as well as several whole polyhedra.

In India, a nuclear polyhedrosis of P.litura was first reported from Delhi by Ramakrishnan and Tiwari (1969). They observed that the diseased larvae did not show any external symptom till a few days before death. Just before death the colour changed to pinkish brown. The incubation period of the disease was 8 to 9 days.

Virus diseases of Amsacta spp.

There is no report available on nuclear polyhedrosis infection of Amsacta. Roberts and Granados (1968) and Mathur (1971) reported a pox-like virus disease in the larvae of the red hairy caterpillar, A.moorei from Rajasthan.

MATERIALS AND METHODS

of 6 mm diameter cut out and placed over a wet filter paper to avoid drying up and inoculated with the virus suspension were supplied to the larvae confined within inverted plastic containers.

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

Histopathology

Fourth instar larvae were inoculated with 10^5 polyhedra per larva as described earlier. At 24 hr intervals up to 120 hr of the treatment, 3 larvae each from the inoculated and control groups were selected at random and used for histological preparations.

The larvae were killed in hot alcoholic Bouin's solution and allowed to soak for approximately 10 minutes, after which the smaller specimens were cut into two and larger ones into three and transferred into cool alcoholic Bouin's fixative for 24 hr (Drake and McEwen, 1959). The fixed specimens were soaked in several changes of 70 per cent ethanol until all the yellow colour disappeared, dehydrated in an ethyl alcohol-butyl alcohol series and embedded in paraffin according to standard procedures. Transverse and

longitudinal sections were cut at 4 to 6 microns. The sections were stained by an azan staining technique developed by Hamm (1966).

Electron micrography:

The electron micrographs of the viruses were prepared by Dr.Jean H.Adams, Insect Pathology Laboratory, Beltsville, Maryland, U.S.A.

Estimation of the chemical constituents of larval body:

In all these studies early fourth instar larvae inoculated with a dosage of 10^5 polyhedra per larva (as described already) were used. Larvae treated similarly but without the inoculum served as control. Samples were taken at 24 hr intervals for a period of 120 hr after treatment. At each occasion 3 samples were taken from treated and control groups, except for carbonydrate and glycogen estimations where 5 samples were taken. The number of larvae per sample varied depending upon the weights. The larvae were starved for 10 hr before analysis. All analyses were done on whole body homogenates only.

All estimations except total nitrogen, uric acid and minerals were done on fresh materials. Total

nitrogen, uric acid and minerals were estimated on dry materials. For this purpose samples drawn as explained above were separately dried to constant weight at 100°C. The dried samples were powdered and mixed and suitable aliquots were used for each estimation.

1. Estimation of total nitrogen and uric acid:

Total nitrogen was estimated on 50 mg aliquots by a micro-Kjeldahl method. The ammonia evolved was absorbed in 4 per cent boric acid solution and titrated against standard sulphuric acid using a mixed indicator of bromocresol green and methyl red (Jackson, 1962). The total nitrogen content was expressed as percentage of dry weight.

For estimation of uric acid 25 mg of the dried and powdered sample was homogenized with distilled water according to Moran (1959) in a glass mortar and the homogenate transferred to a centrifuge tube. The proteins were precipitated with tungstic acid according to Haden (1923). The precipitate was removed by centrifugation and the supernatant made up to a known volume was used for estimation.

Uric acid was determined by the method of Brown (1945). The procedure outlined by Hawk et al.

(1954) was followed except that only 1 ml of the tungstic acid filtrate was used for each determination and the amount of other reagents adjusted accordingly. The optical density was read in a Spectronic 20 spectrophotometer at 520 mμ wavelength. An appropriate blank was run simultaneously. The uric acid content was calculated by referring to a standard curve and expressed as percentage of dry weight. Pure uric acid (BDH, England) was used to prepare the uric acid standard as outlined by Hawk et al. (1954).

2. Estimation of total carbohydrate and glycogen:

The analyses were made on fresh materials. The total carbohydrates and glycogen were extracted by the method of Crompton and Birt (1967).

Larvae were washed in water, dried on filter paper and weighed. Each sample was then homogenized in ice-cold 0.3 N perchloric acid for 2 minutes in a glass mortar. The homogenate was kept at 5°C overnight (Orr, 1964) instead of 10 minutes adopted by Crompton and Birt (1967). Insoluble matter was removed by centrifuging for 10 minutes at 5000 rpm and washed twice in ice cold 0.3 N perchloric acid by redispersion and centrifugation. The three supernatants

were combined into an acid extract and made up to a known volume with distilled water.

The total carbohydrates was determined in the acid extract colorimetrically by the anthrone method of Fairbairn (1953) with 1 ml sample and 5 ml anthrone. The colour was read in a Klett-Summerson photoelectric colorimeter with No.66 filter. A suitable blank was run. A standard curve was prepared for glucose (BDH, England). The colorimetric readings were referred to the standard curve and the carbohydrate content was calculated as mg glucose/g body weight.

Glycogen was precipitated from the acid extract (10 ml) according to Roe et al. (1961) by the addition of 4 volumes of 95 per cent (v/v) ethanol. The mixture was kept overnight at 5°C. The precipitate was sedimented by centrifugation at 5°C, redispersed in fresh ethanol and resedimented. The washed pellets were dried in a desiccator and then dissolved in a known volume of distilled water. Aliquots (1 ml) were estimated colorimetrically for the glycogen content by the anthrone method of Fairbairn (1953). Oyster glycogen (BDH, England) was used as the standard. The colour was read in a Klett-Summerson photoelectric colorimeter with No.66 filter. The glycogen content was calculated

by referring to the standard curve and was expressed as mg glycogen/g wet weight.

3. Estimation of total fat:

Analyses were made on fresh larvae. Groups of larvae were homogenized in chloroform-methanol (2:1 v/v) after the method of Folch et al. (1957) and extracted at 60°C for 5 minutes (Orr, 1964). The insoluble material was separated by centrifuging at 5000 rpm for 10 minutes. The supernatant liquid was collected in a separating funnel. The precipitate was resuspended in 5 ml of chloroform-methanol and the extraction procedure repeated. The supernatant was added to the original one in the separating funnel and partitioned against 3 per cent (w/v) aqueous sodium chloride (Schaeffer, 1968). The chloroform phase was drawn off and the aqueous phase washed twice with chloroform. The combined chloroform phase was dried under reduced pressure and the lipid residue was dissolved in a minimum quantity of diethyl ether and transferred to a weighing bottle. The ether was allowed to evaporate completely and the dry lipid residue was weighed. The total fat content was expressed as mg/g wet weight of larvae.

4. Estimation of nucleic acids and protein:

Estimations were done on fresh whole body homogenates only. The preliminary separation of nucleic acids and protein from other fractions was done following the method of Orr (1964) and Price (1969). Groups of larvae (3 to 20) were homogenized in a glass mortar at 0 to 2°C with ice cold 0.4 N perchloric acid and extracted overnight at 5°C. The homogenate was then centrifuged at 5000 rpm for 25 minutes at 5°C in a refrigerated centrifuge. The supernatant was discarded and the residue was extracted twice with 0.4 N perchloric acid and centrifuged as before. This was followed by extraction with 90 per cent ethanol and twice with chloroform-methanol (2:1 v/v) at 60°C for 5 minutes to remove the lipids. Each homogenate was centrifuged at 5000 rpm for 10 minutes. The lipid free residue was dried in a desiccator over $CaCl_2$.

The dry tissue was suspended in 0.5 N perchloric acid and the tubes were heated at 90°C for 20 minutes in a water bath (Orr, 1964; Kilgore and Painter, 1964). After this the suspension was centrifuged and the supernatant solution was analysed for nucleic acids. The residue was reserved for protein extraction (Orr, 1964; Price, 1969).

i) Estimation of DNA:

DNA was determined by the diphenylamine method of Burton (1956) in 3 ml final volume. Sodium salt of calf thymus gland DNA (BDH, England) was used as the standard. The optical density was read at 600 mμ in a Spectronic 20 spectrophotometer. The total DNA content was calculated by referring to the standard curve and expressed as μg DNA/mg body weight.

ii) Estimation of RNA:

RNA was determined in the perchloric acid extract by the Orcinol method of Ceriotti (1955) in 10 ml final volume. The optical density was read at 675 mμ in a Spectronic 20 spectrophotometer. Yeast RNA (BDH, England) was used as the standard. The total RNA was calculated by referring to the standard curve and expressed as μg RNA/mg body weight.

iii) Estimation of protein:

Protein was extracted from the perchloric acid insoluble residue according to Orr (1964). The dry residue was homogenized in 1 N NaOH and heated for 15 minutes at 100°C. This was diluted to 0.1 N NaOH by the addition of distilled water. The undissolved

material was removed by centrifugation at 5000 rpm for 20 minutes. The supernatant fluid was assayed for protein by the method of Lowry et al. (1951). Bovine serum albumin (Miles Laboratories, Kankakee, U.S.A.) was used as the standard. The optical density was read at 750 mμ in a Spectronic 20 spectrophotometer. The protein content was calculated by referring to the standard curve and expressed as mg protein/g wet weight.

5. Estimation of minerals

Ca, Mg, Na, K and P were estimated in ternary acid extract of larval material.

Acid digestion: Larval samples, dried and powdered, 0.5 g in weight were digested over a sand bath with 15 ml of ternary acid mixture consisting of nitric acid, perchloric acid and sulphuric acid in the proportion 10:1:4 till the solution became colourless and clear. The solution was cooled, filtered and the filtrate made up to 100 ml and this extract was used for the estimation of Ca, Mg, Na, K and P. Three samples each from treated and untreated group for each interval were digested.

i) Calcium:

Calcium was estimated by titration against 0.02 N versenate. To a 5 ml aliquot of the ternary acid extract, 10 ml of 10 per cent KOH and a pinch of murexide indicator powder were added and titrated against 0.02 N versenate until the vine red colour turned to violet. The calcium content was expressed as percentage of dry weight.

ii) Calcium + Magnesium:

To 5 ml of the acid extract, 2 ml of a buffer solution (NH_4OH + NH_4Cl - pH 10) and a few drops of eriochrome black T indicator were added. It was titrated against 0.02 N versenate until the colour changed from red to blue.

The titre value corresponds to the Ca + Mg present in the aliquot and Mg concentration was calculated from the difference between the titre values for Ca + Mg and Ca.

iii) Sodium and Potassium:

These minerals were estimated using an ELL-Flame photometer as described by Jackson (1962). A suitable aliquot of the ternary acid extract was

neutralized by the addition of ammonia and used for the flame photometric estimation. By referring to standard curves, the quantity of Na and K were calculated and expressed as percentage of dry weight.

iv) Total phosphorus:

The vanadomolybdophosphoric yellow colour method of Jackson (1962) was employed. To a 5 ml aliquot of the acid extract 5 ml of vanadomolybdate reagent was added to develop the yellow colour, the volume made up to 25 ml and the colour intensity was read after 30 minutes in a Coleman Nephocolorimeter at 470 mμ. An appropriate blank was run simultaneously. The total phosphorus content was calculated by referring to a standard curve and expressed as percentage of dry weight.

6. Total haemocyte count:

Early fourth instar larvae were inoculated with 10^5 polyhedra/larva as described already. Haemolymph samples were drawn at 24, 48, 72 and 120 hr after treatment. Haemocytes of 10 larvae were estimated at each interval. The larvae were killed by immersing in hot water at 55° to 60°C for 2 to 3 minutes (Jones, 1962). Blood was withdrawn by cutting a proleg on the

sixth abdominal segment with a fine scissors into a Thoma white cell pipette and diluted with 2 per cent versene saline (Patton and Flint, 1959). The pipette was shaken for several minutes and the first three drops were discarded. A haemocytometer (improved double Neubauer ruling) chamber was filled and the haemocytes were counted at a magnification of 450 x as outlined by Jones (1962) and the number of haemocytes per cubic millimetre calculated with the formula

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

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

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

A survey conducted in and around Coimbatore, Tamil Nadu during 1968-69 revealed the occurrence of nuclear polyhedrosis in three species of lepidopterous pests viz., the red hairy caterpillar, Amsacta albistriga Walker (Arctiidae), the gram pod borer, Heliothis armigera Hubner (Noctuidae) and the tobacco caterpillar, Spodoptera litura Fabricius (Noctuidae). Some general observations were made on the nuclear polyhedroses of A.albistriga and H.armigera while, detailed studies were made on the nuclear polyhedrosis of S.litura. Details and results of these studies are presented in the following sections:

NUCLEAR POLYHEDROSIS OF THE RED HAIRY CATERPILLAR, Amsacta albistriga W.

Caterpillars of A.albistriga showing characteristic signs and symptoms of a polyhedrosis were collected from groundnut (Arachis hypogaea) fields at Pollachi in Coimbatore District. Microscopic examination of haemolymph and tissue smears showed the presence of polyhedra. The inclusion bodies were separated by maceration of affected caterpillars in distilled water

and purified by filtration and centrifugation. Pathogenicity tests were made on 8 to 10 days old Amsecta caterpillars collected from an apparently disease free area. A total of 125 larvae, in batches of 25, were inoculated by feeding them for 48 hr on groundnut leaves contaminated with a heavy suspension of polyhedra in 0.1 per cent teepol. Seventy five larvae of the same age group fed with groundnut leaves which were sprayed with 0.1 per cent teepol alone served as control. After 48 hr, both control and treated larvae were transferred to fresh untreated leaves and thereafter fresh leaves were provided every day. Observations were recorded on the signs and symptoms and mortality of the larvae. Out of the 125 larvae inoculated with the virus, 98 (78.4 per cent) died of polyhedrosis. No mortality was observed in the control group.

Signs and symptoms:

Infected larvae exhibited loss of appetite and became sluggish in movement within 5 to 6 days after ingestion of the polyhedra. The larvae assumed a dirty brown colour. Death occurred in 8 to 10 days with liquefaction of internal tissues. At death the cuticle

Fig. 1. Technique of inoculation of larvae:

A. Castor leaf showing the paper gum tape strips pasted over the lamina.

B. Larvae released over the inoculated spot on the leaf and covered with penicillin vials.

C. Castor leaf showing the inoculated spots (arrows) fed by the larvae.

Fig. 1

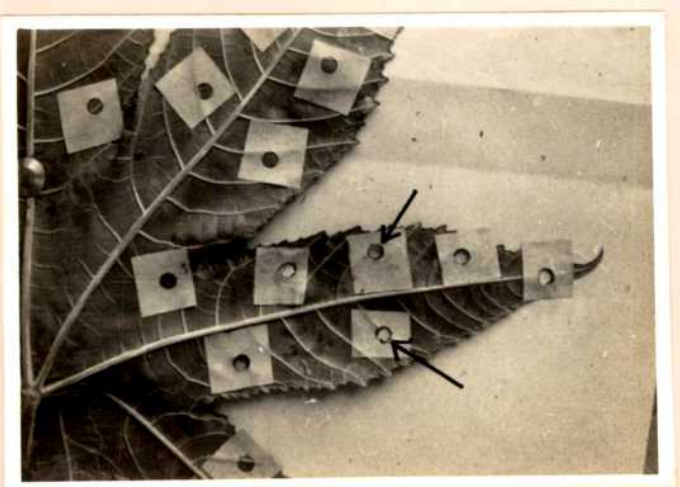


Fig. 2. Larva of the red hairy caterpillar, Amsacta albistriga W. died of nuclear polyhedrosis.

Fig.2

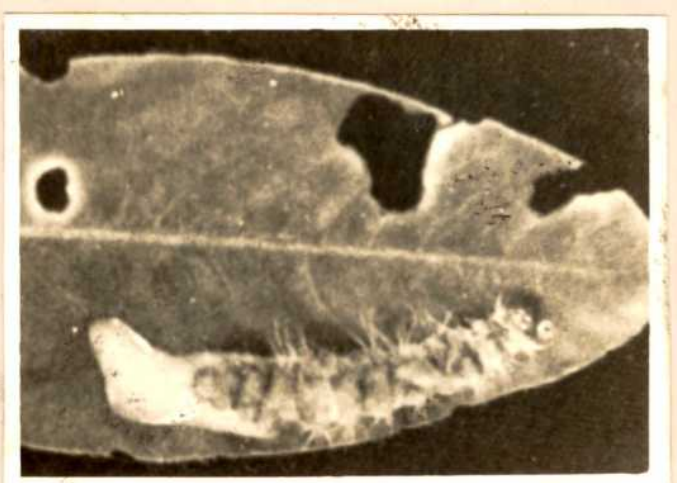


Fig. 3. Electron micrograph of polyhedra isolated from Amsacta albistriga 24900x.

Fig.3

Fig. 4. Electron micrograph of sections of polyhedra isolated from Amsacta albistriga showing the arrangement of virus rods (arrows). 95506x

Fig.4

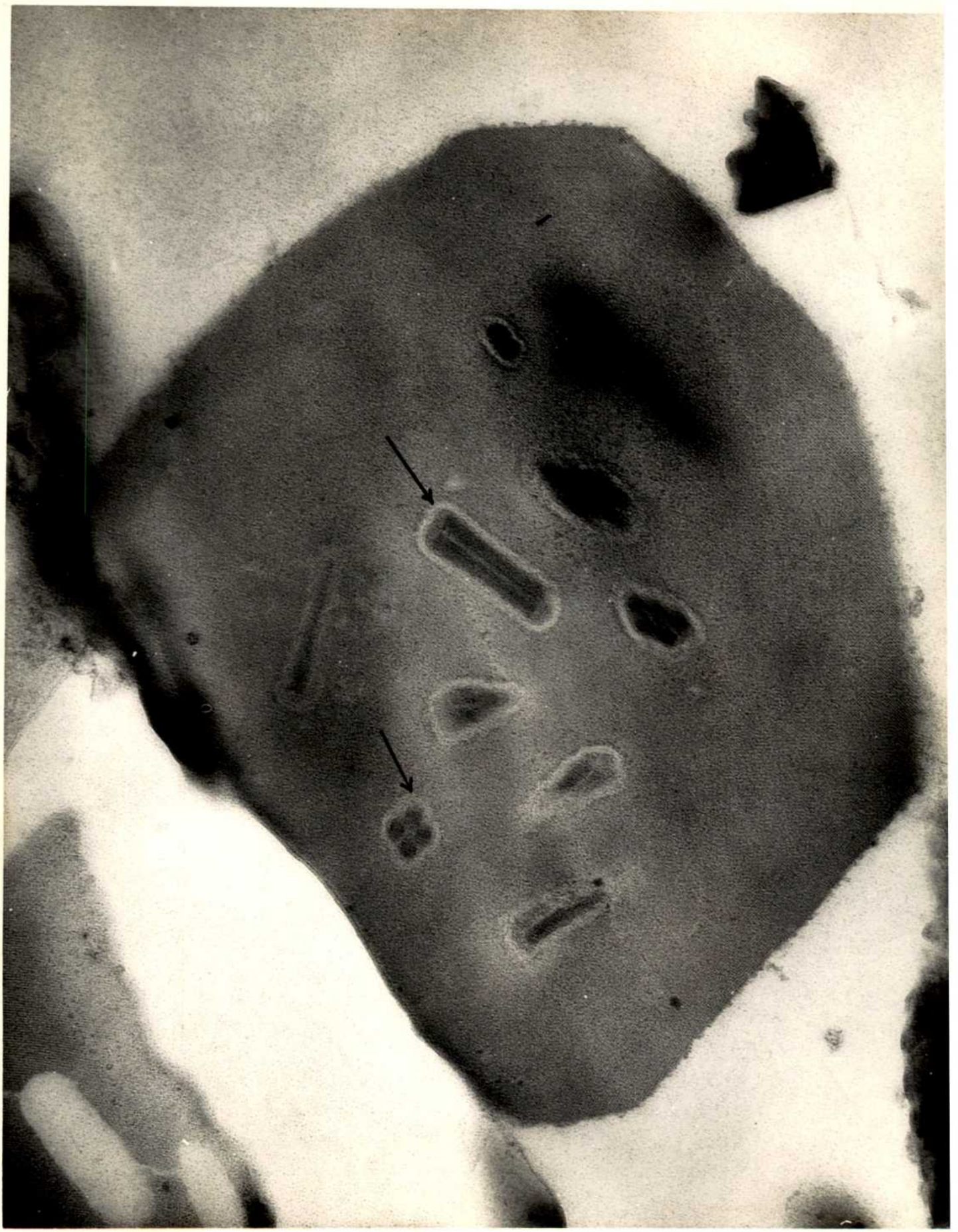


Fig. 5. Electron micrograph of carbon replicas of polyhedra isolated from Amsacta albistriga. 23900x

Fig.5

Fig. 6. Larva of the gram pod borer, Heliothis armigera died of nuclear polyhedrosis.

Fig.6

Fig. 7. Electron micrograph of polyhedra isolated from Heliothis armigera 24900x.

Fig.7

Fig. 8. Electron micrograph of sections of polyhedra isolated from Heliothis armigera showing the arrangement of virus rods (arrows). 67422x

Fig.8

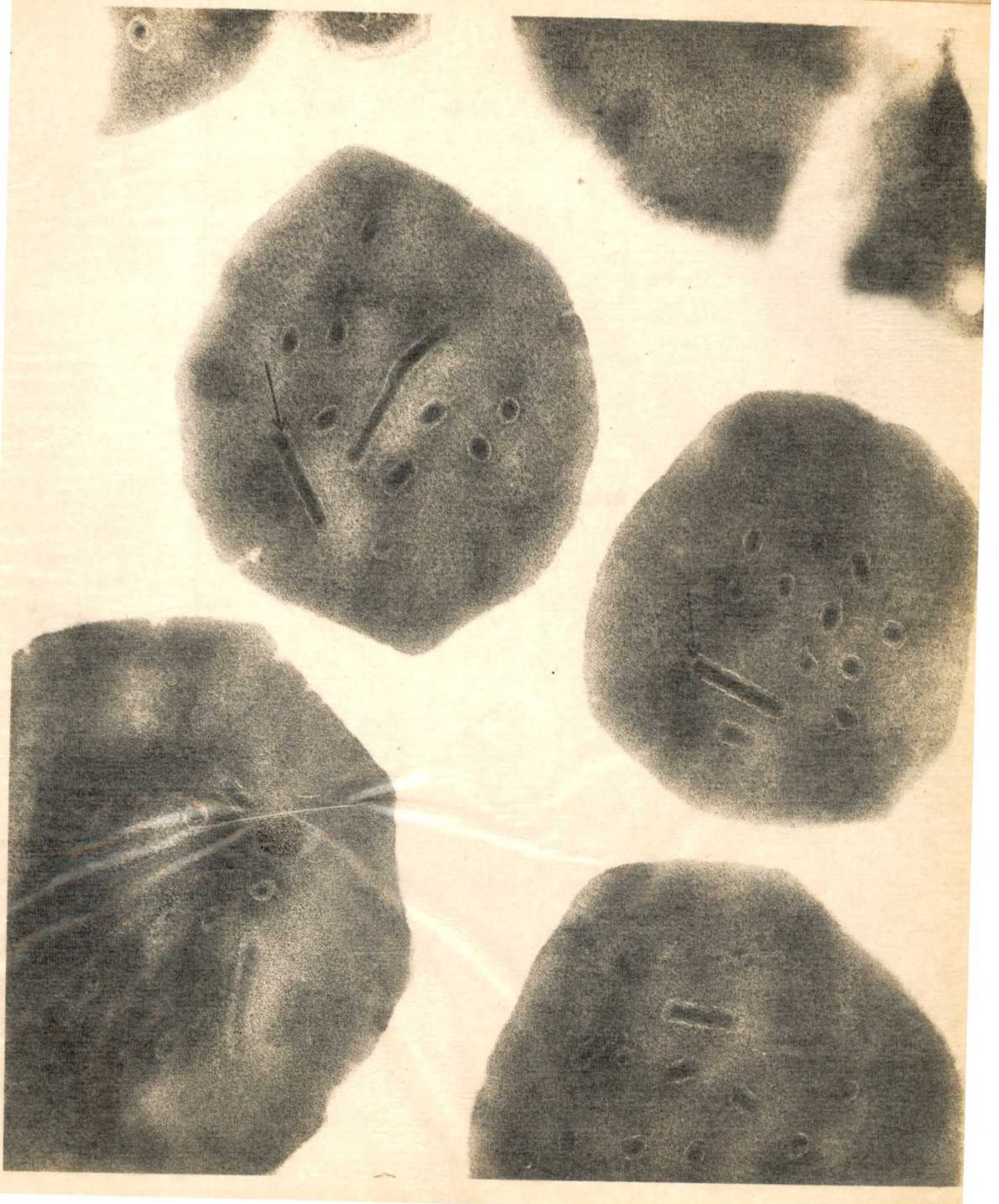


Fig. 9. Electron micrograph of carbon replicas of polyhedra isolated from Heliothis armigera. 28722 x

Fig.9

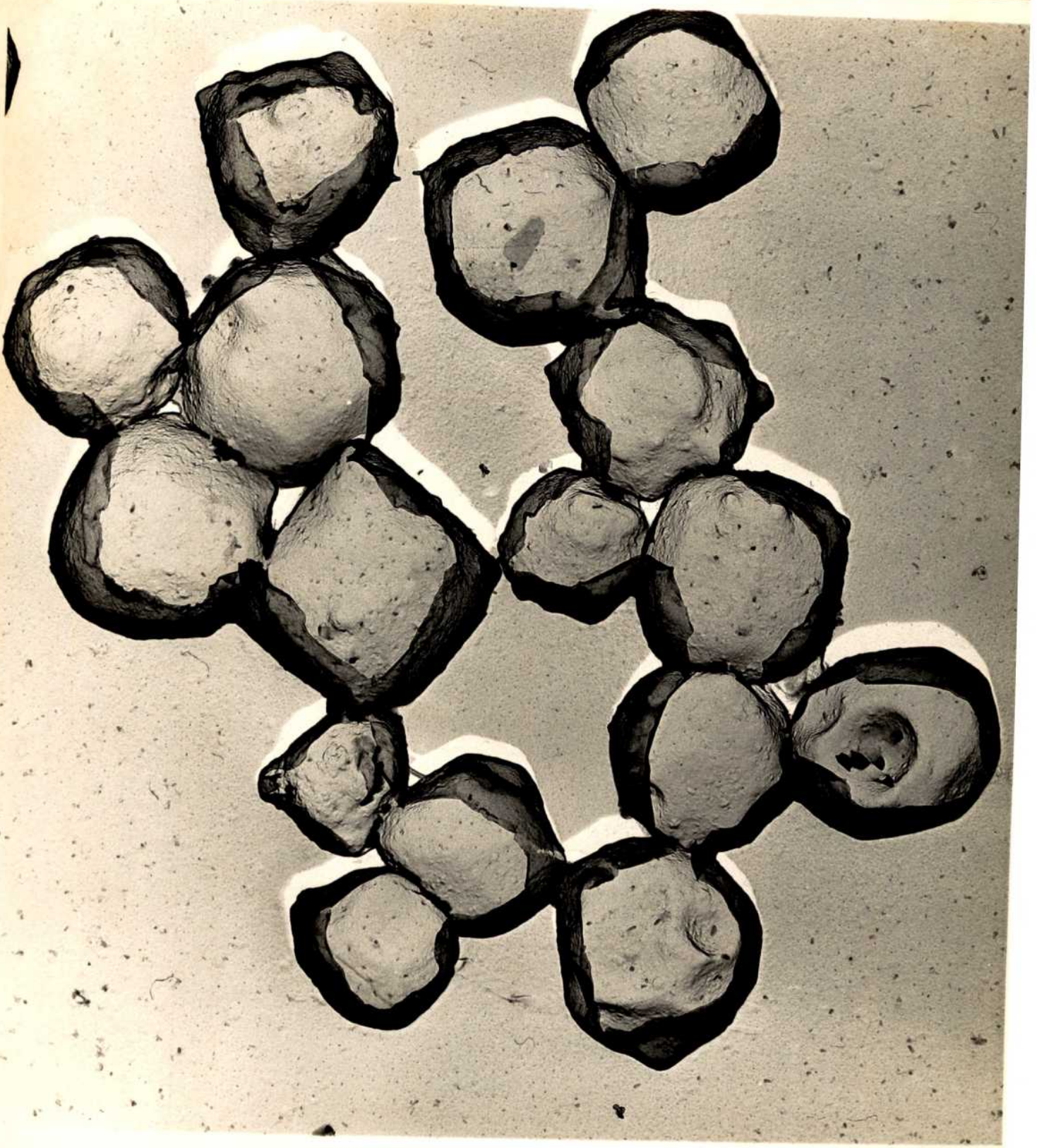


Fig. 10. Healthy (H) and diseased (D) larvae of Spodoptera litura 3 days after ingestion of polyhedra (inoculated in the early fourth instar). Healthy and diseased larvae now in the fifth instar. Note the bloated appearance and slight pinkish tinge of the diseased larva.

Fig. 11. Healthy (H) and diseased (D) larvae of S.litura 4 days after ingestion of polyhedra (inoculated in the early fourth instar). Healthy larva in the sixth instar but the diseased larva still in the fifth instar. Note the intense pink colouration of the diseased larva.

Fig.11

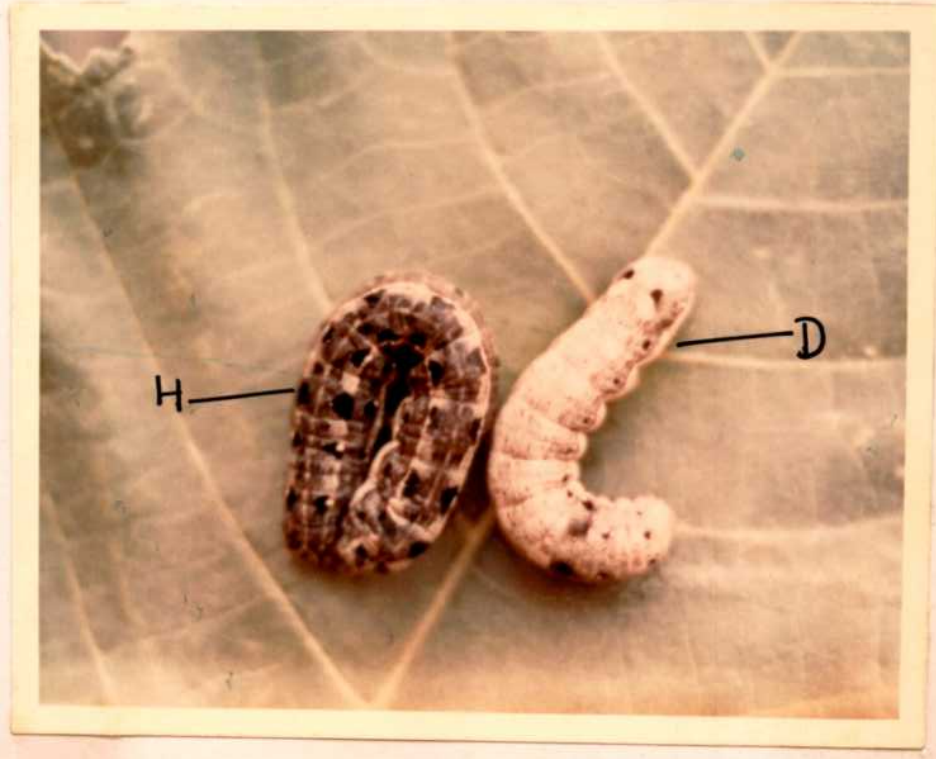


Fig. 12. Healthy and diseased larvae of Spodoptera litura 5 days after ingestion of polyhedra (inoculated in the early fourth instar). Healthy larva in the late sixth instar but the diseased larva still in the fifth instar. Note the pink colouration and reduced size of the diseased larva.

Fig.12

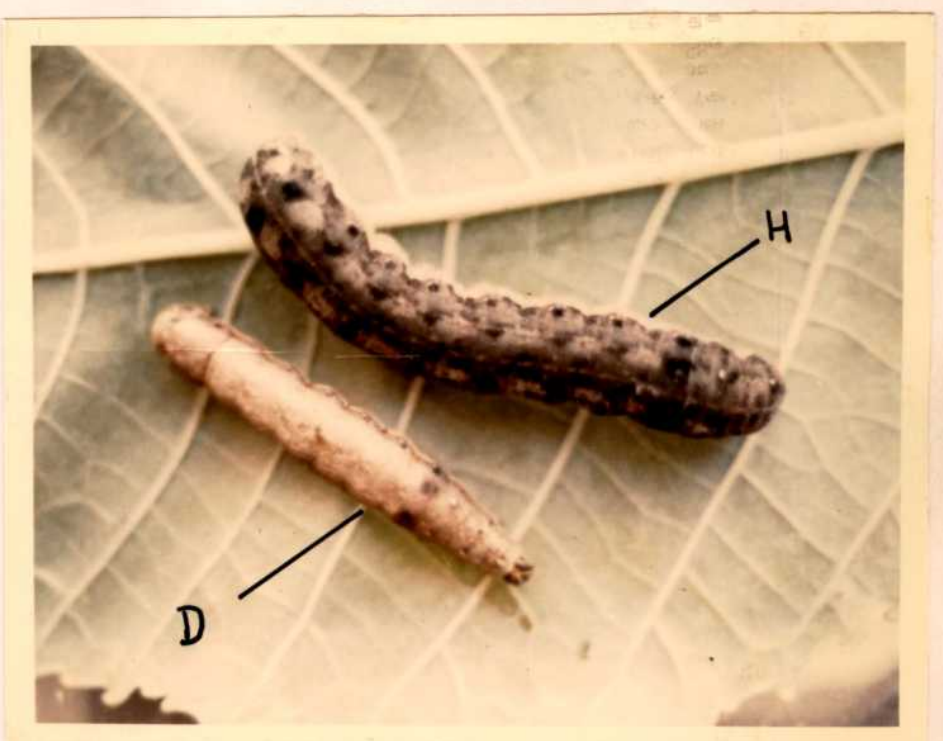


Fig. 13 and 14. Larvae of Spodoptera litura infected in the fifth instar showing the bloated appearance and pink colouration.

Fig. 13

Fig. 14

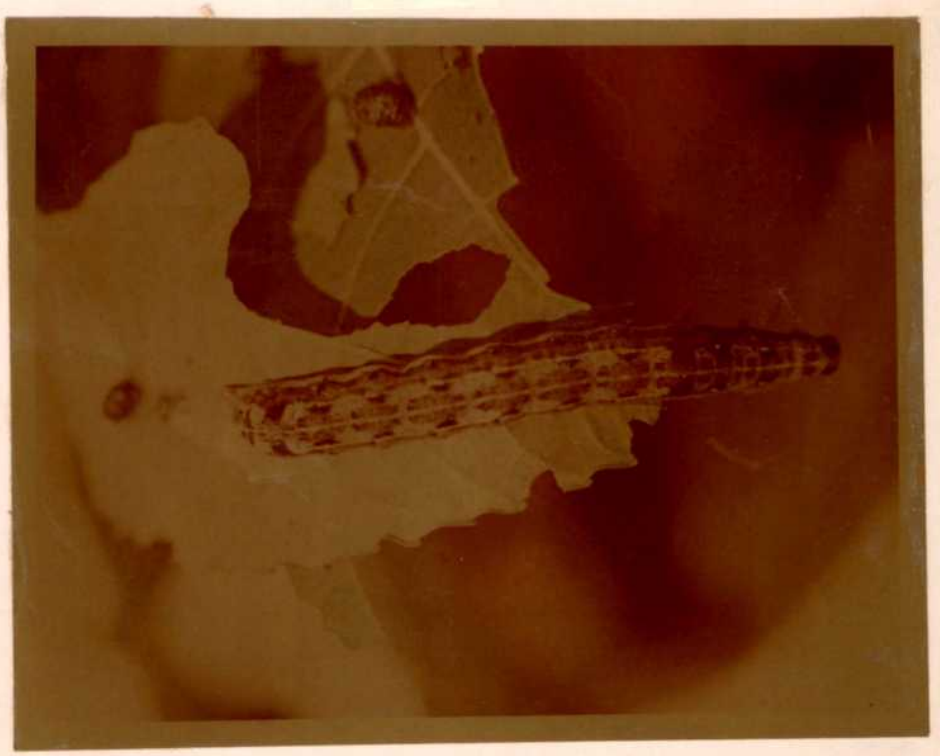


Fig. 15. Larva of Spodoptera litura which ingested the polyhedra in the second instar and died of nuclear polyhedrosis in the third instar.

Fig. 16. Larva of Spodoptera litura which ingested the polyhedra in the third instar and died of nuclear polyhedrosis in the fourth instar.

Fig.15

Fig.16

Fig.17 and 18. Larvae of Spodoptera litura died of nuclear polyhedrosis showing the characteristic symptoms.

Fig.18

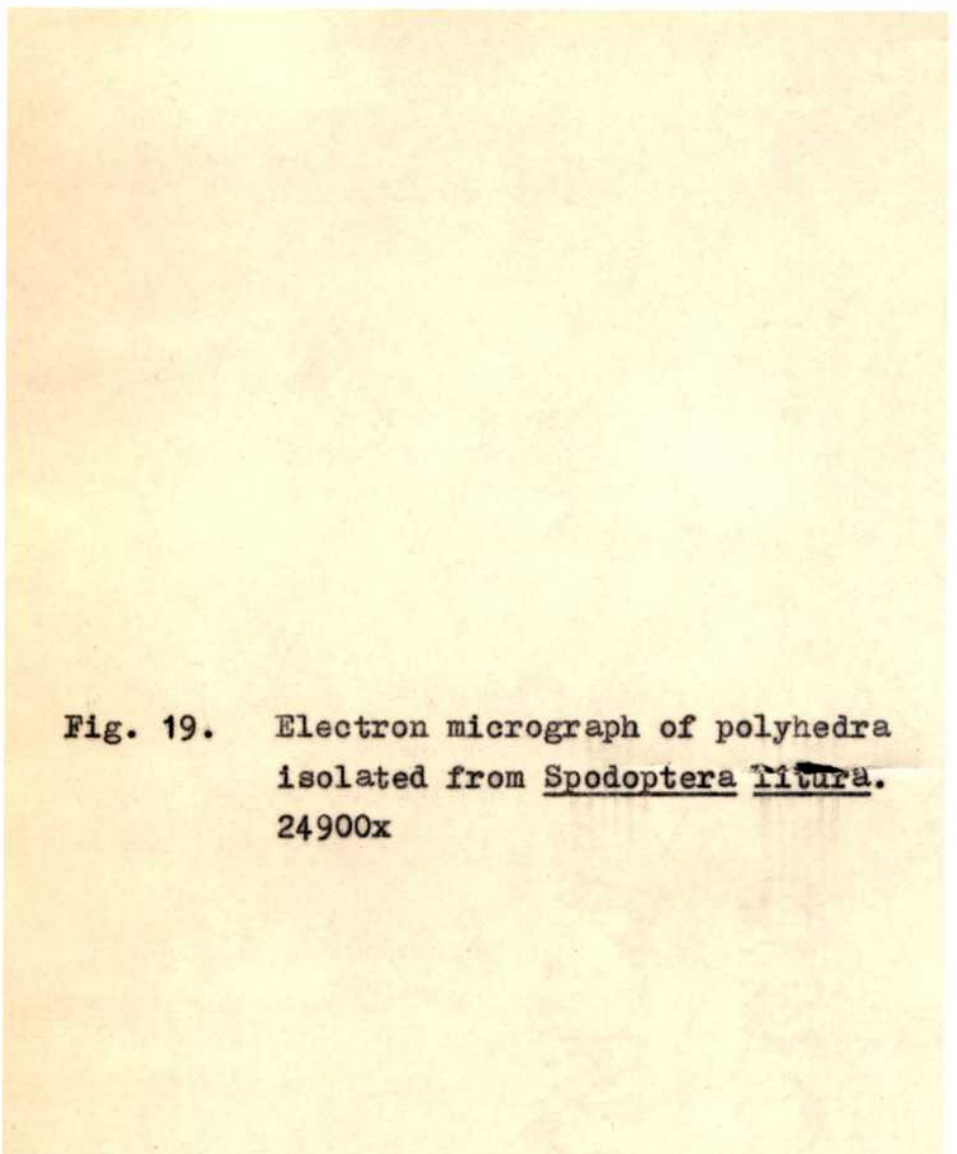


Fig. 19. Electron micrograph of polyhedra isolated from Spodoptera litura. 24900x

Fig.19

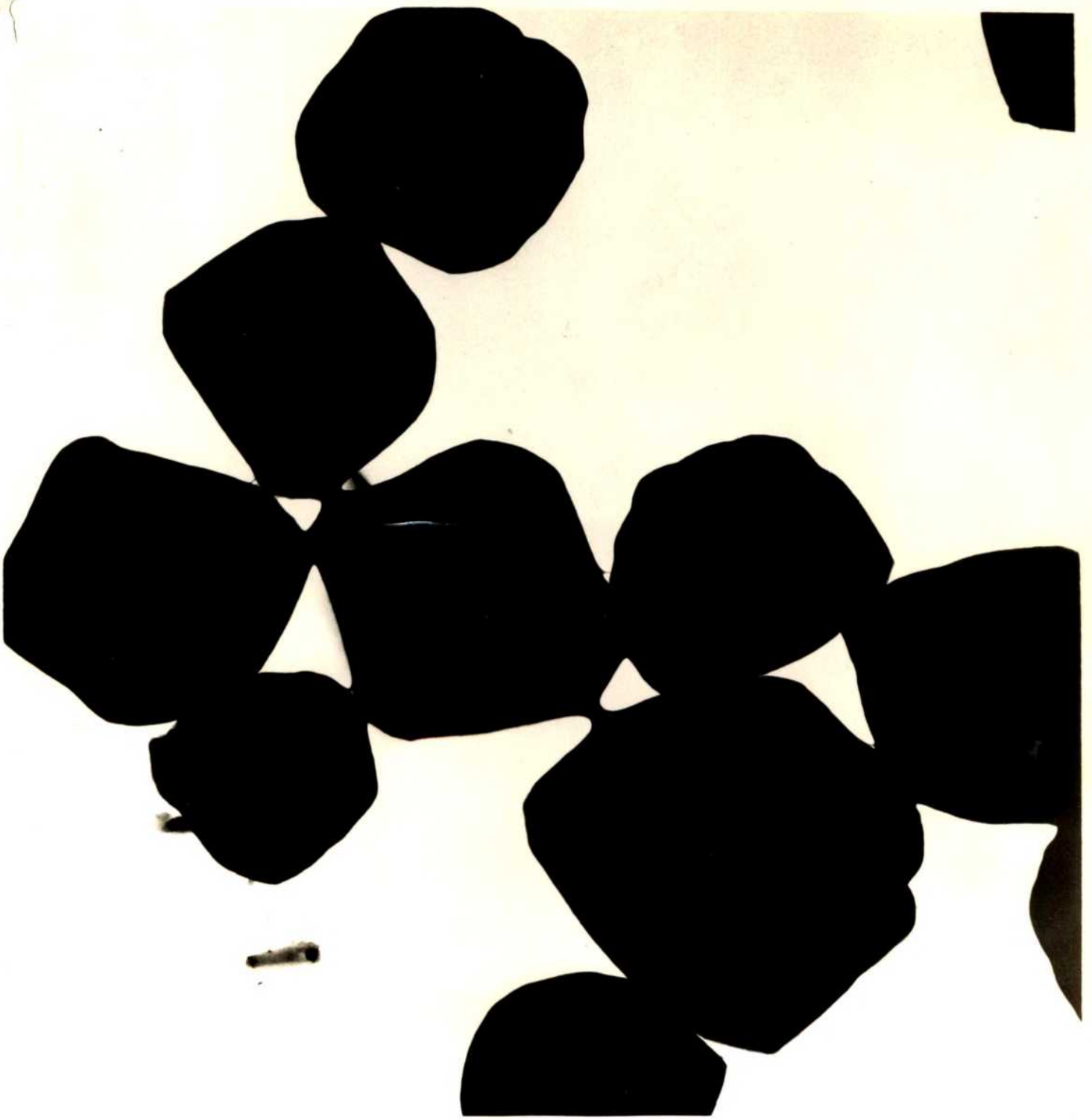
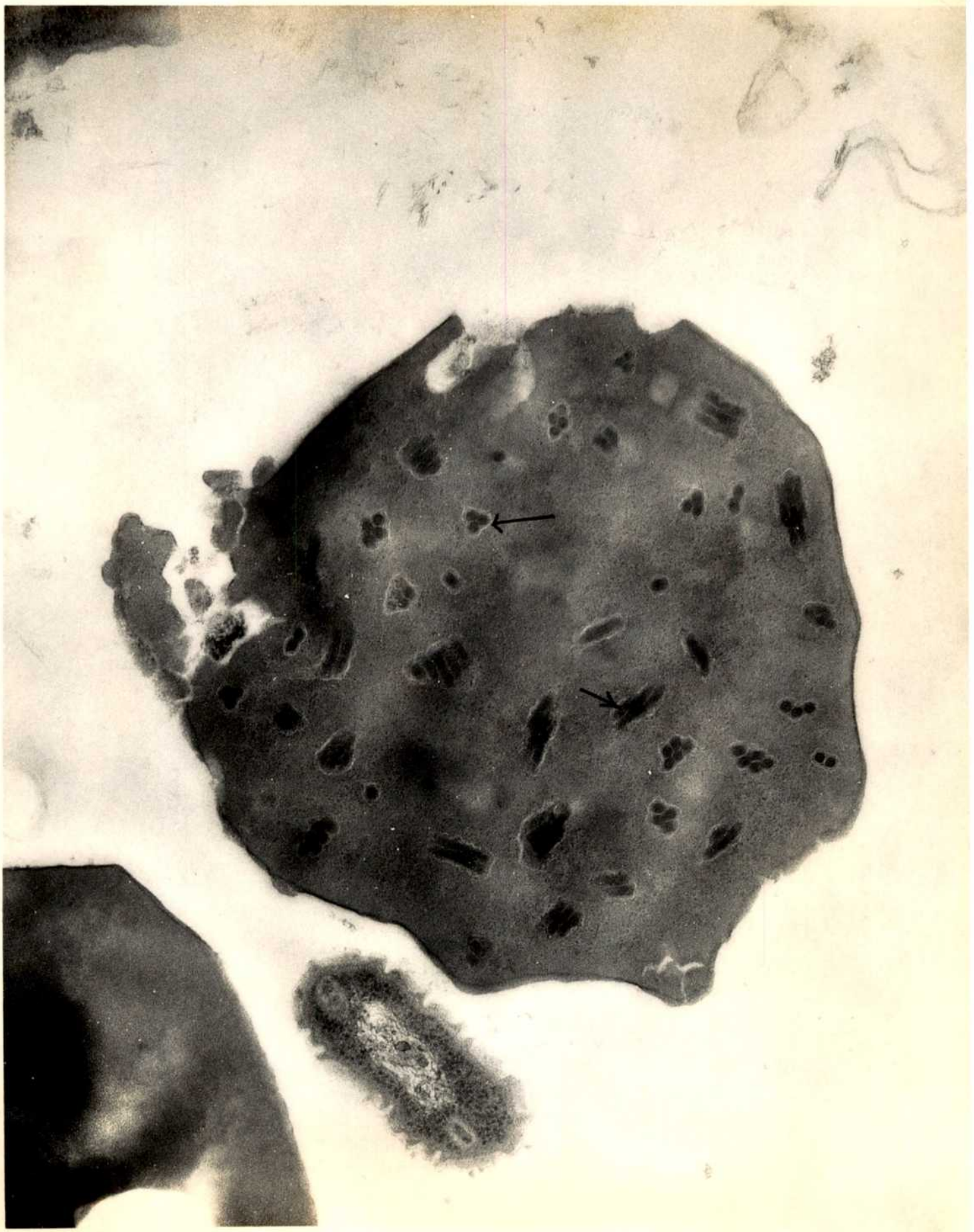


Fig. 20. Electron micrograph of sections of polyhedra isolated from Spodoptera litura showing the arrangement of virus rods (arrows). 55952x

Fig.20



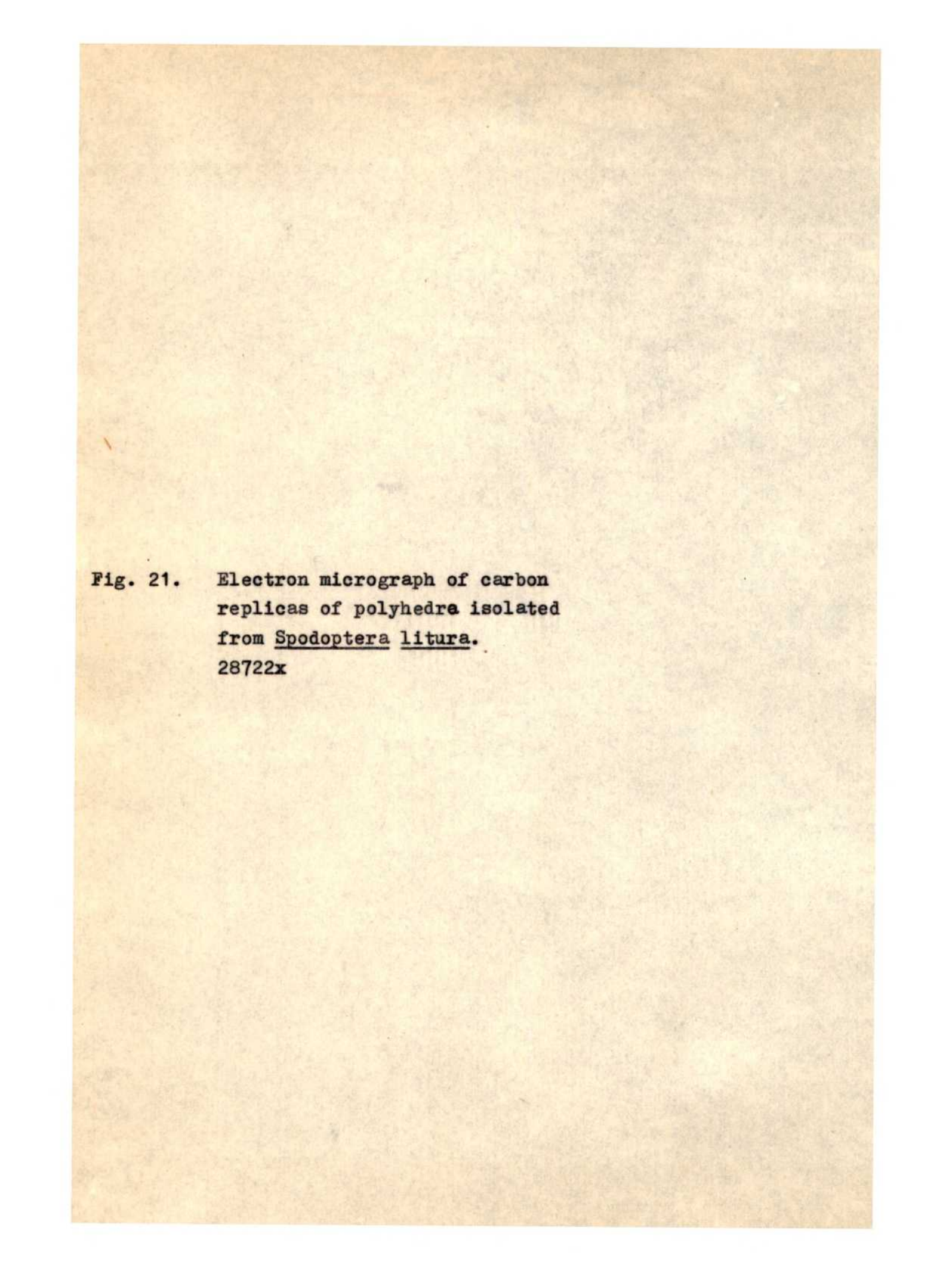


Fig. 21. Electron micrograph of carbon replicas of polyhedra isolated from Spodoptera litura.
28722x

Fig.21

Fig. 22. Wet weight of healthy and NPV infected larvae of Spodoptera litura at different periods after inoculation.

Fig. 23. Dry weight of healthy and NPV infected larvae of Spodoptera litura at different periods after inoculation.

Fig.22

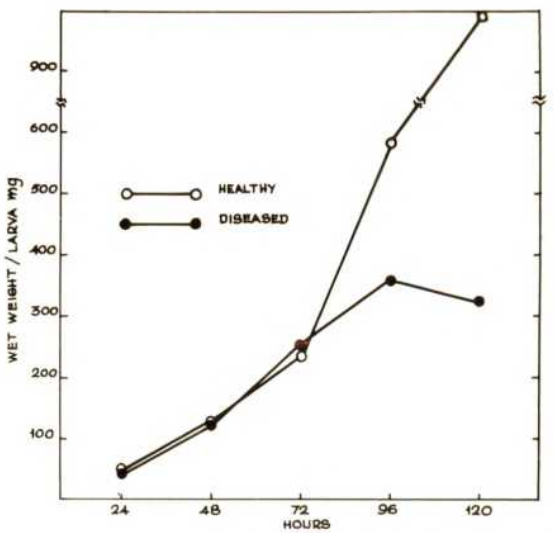

WET WEIGHT / LARVA mg
900
600
500
400
300
200
100
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig.23

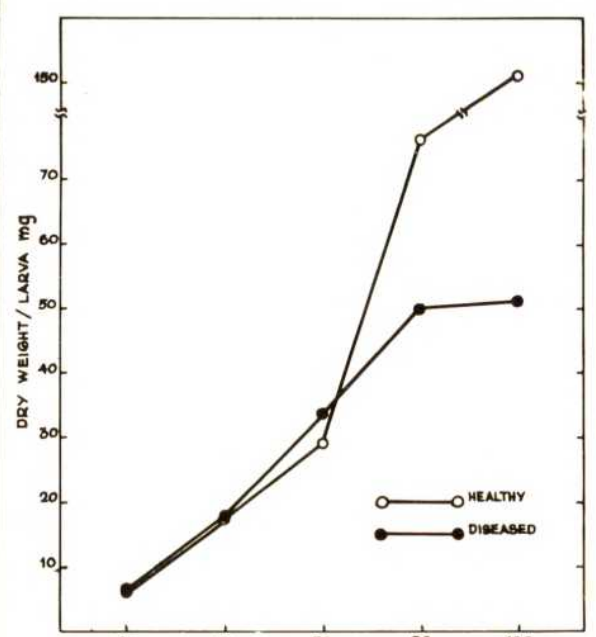

DRY WEIGHT / LARVA mg
150
70
60
50
40
30
20
10
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig. 24. Mean length of healthy and NPV infected larvae of Spodoptera litura at different periods after inoculation.

Fig. 25. Area of castor leaf consumed by healthy and NPV infected larvae of S.litura at different periods after inoculation.

Fig.24

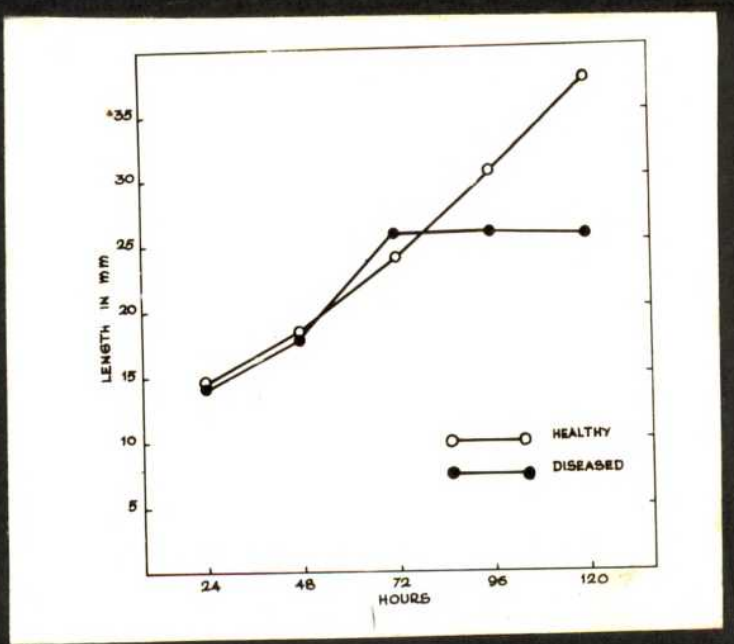

LENGTH IN mm
35
30
25
20
15
10
5
24
48
72
96
120
HOURS
HEALTHY
DISEASED

Fig.25

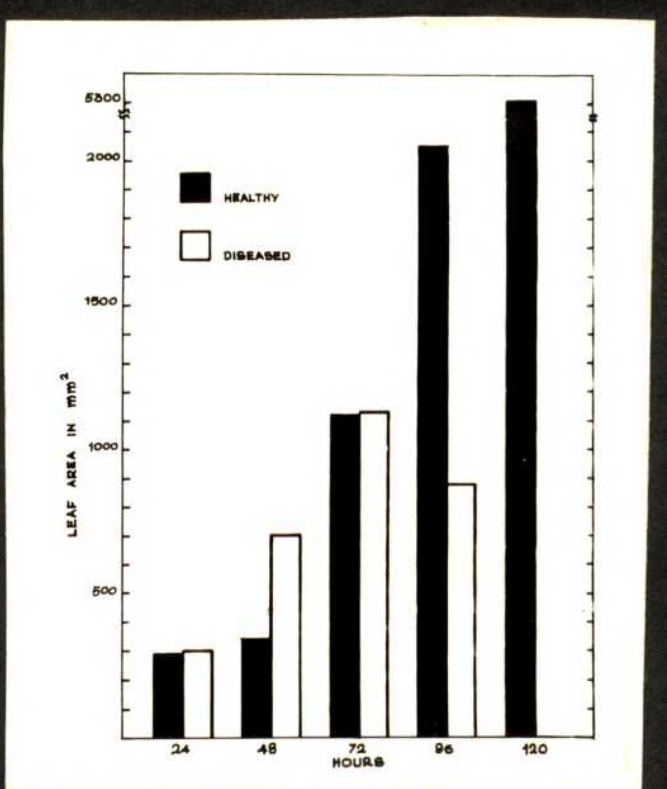

LEAF AREA IN mm²
2500
2000
1500
1000
500
24
48
72
96
120
HOURS
HEALTHY
DISEASED

Fig. 26. Section of NPV infected larva of Spodoptera litura 72 hr after inoculation showing the polyhedral formation in hypodermis (H), fat body (F) and tracheal matrix (T). 320x.

Fig. 27. Section of NPV infected larva of S.litura, 96 hr after inoculation, showing polyhedra in different tissues. 320x

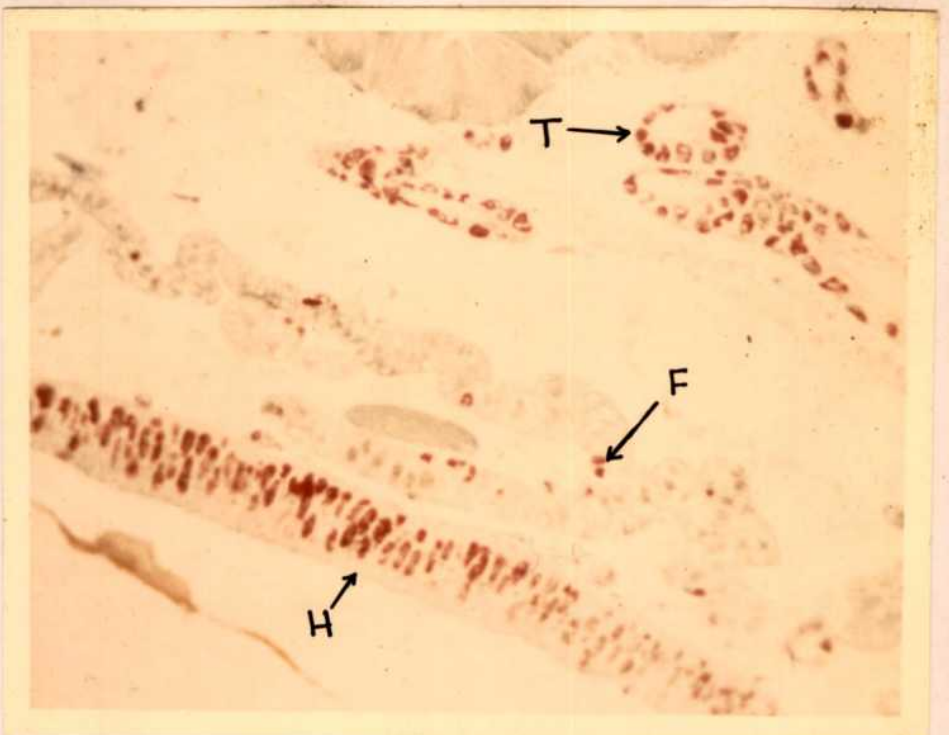


Fig.26

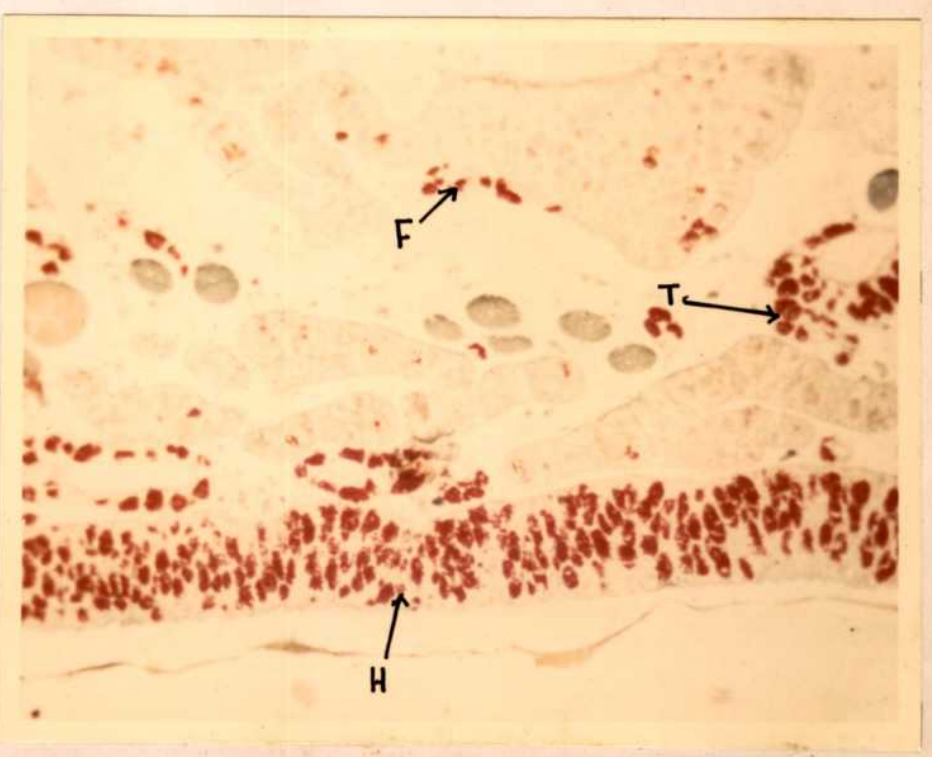


Fig.27

Fig. 28. Section of NPV infected larva of Spodoptera litura 120 hr after inoculation showing advanced stages of infection in hypodermis (H), trachea (T), fat body (F) and muscle (M). 320x

Fig. 29. Section of hypodermis of NPV infected larva of S.litura 120 hr after inoculation showing the advanced stage of infection; P - polyhedra. 900x

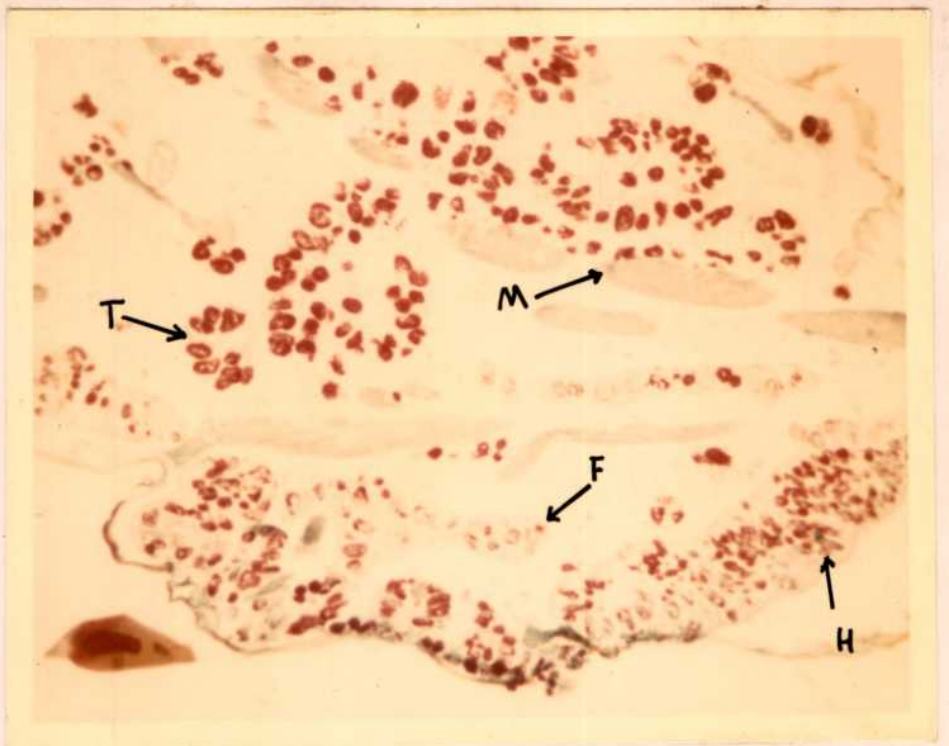


Fig.28

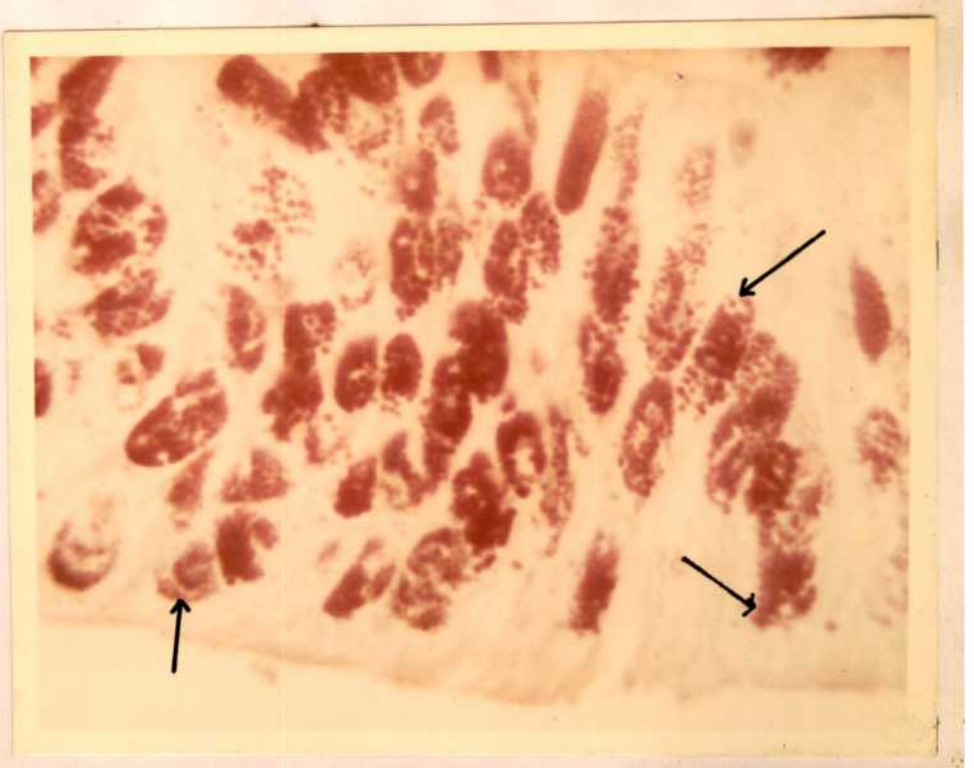


Fig.29

Fig. 30. Section of fat body of NPV infected larva of Spodoptera litura 120 hr after inoculation showing advanced stage of infection.
P - polyhedra. 180x

Fig. 31. Section of trachea of NPV infected larva of S.litura 120 hr after inoculation showing advanced stage of infection. 810x

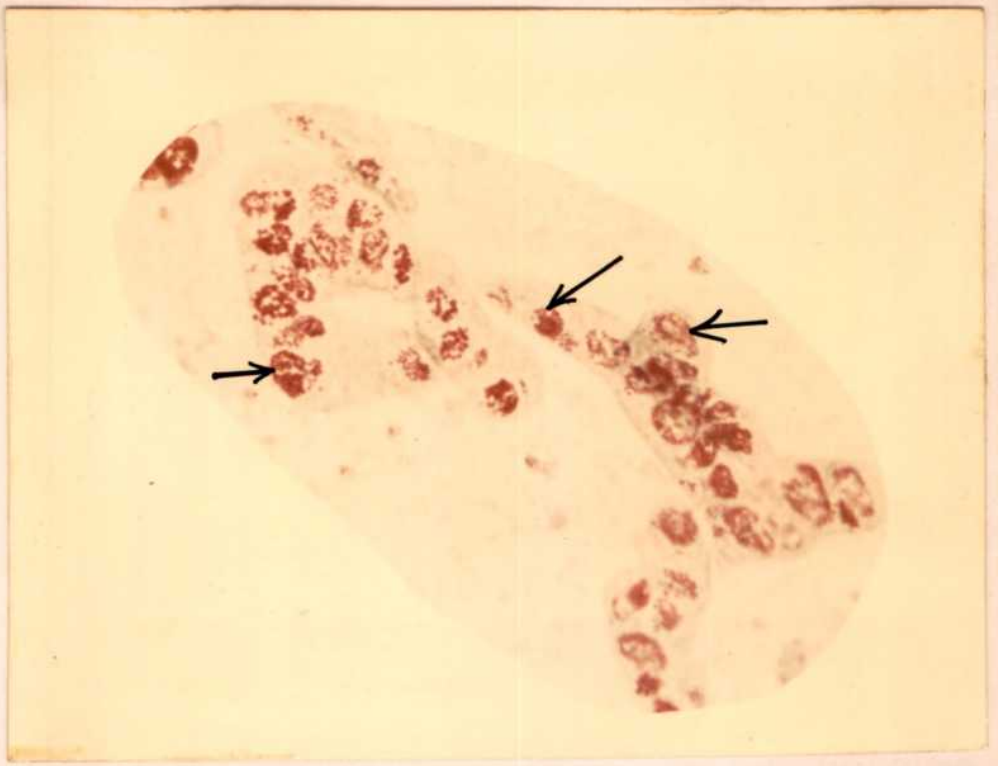


Fig.30

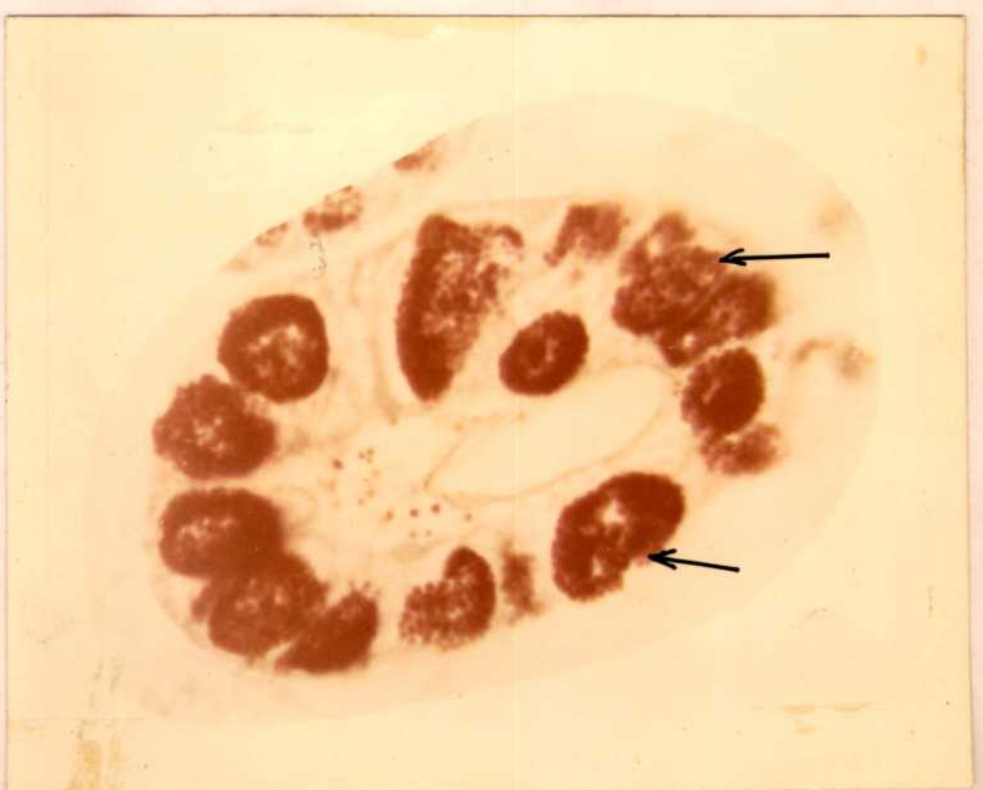


Fig.31

Fig. 32. Section of hypodermis of healthy and NPV infected larvae of Spodoptera litura 48 hr after inoculation. 1000x
A - Healthy; B - Diseased
Note the hypertrophied nuclei (HN) and the virogenic stroma (VS).

Fig. 33. Sections of hypodermis of healthy and NPV infected larvae of S.litura 72 hr after inoculation. 1000x
A - Healthy; B - Diseased
Note the thickened hypodermis with fully developed polyhedra (P) inside the hypertrophied nuclei.

Fig.32

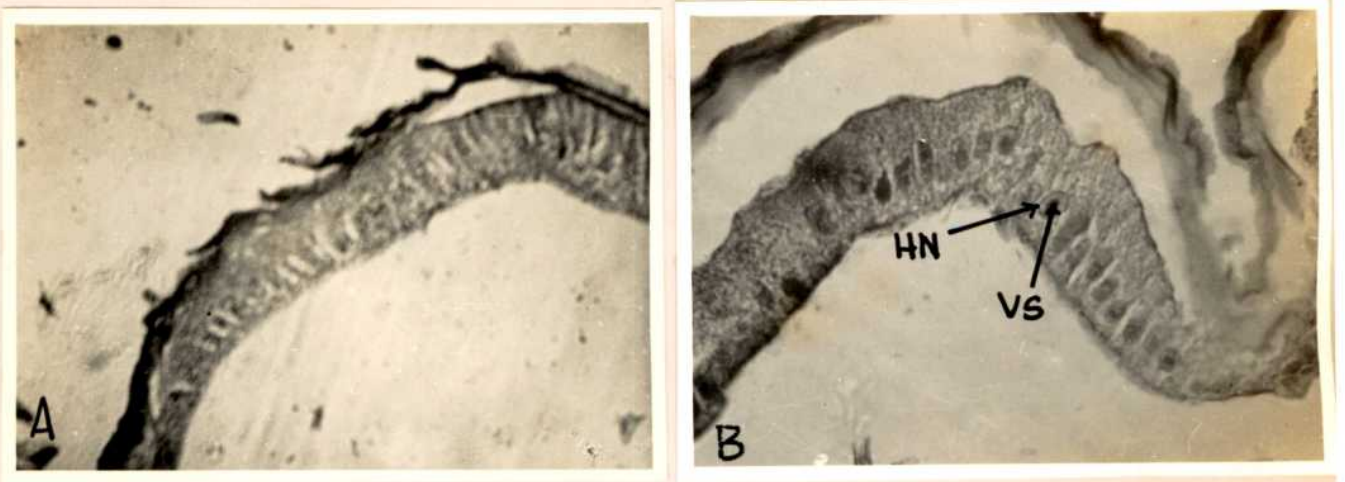


Fig.33

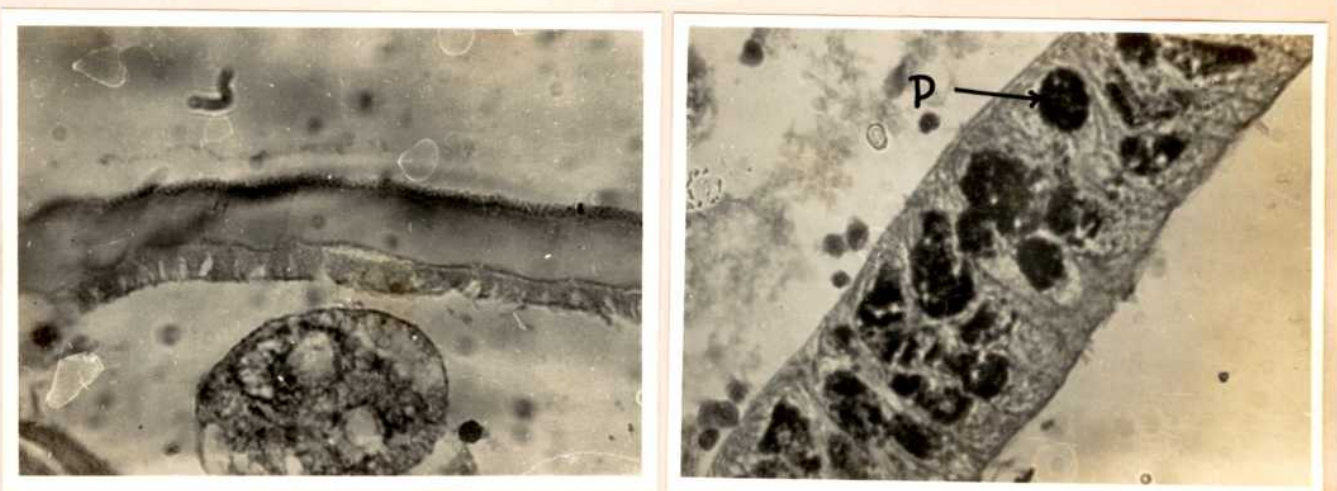


Fig. 34. Sections of hypodermis of healthy and NPV infected larvae of Spodoptera litura, 96 hr after inoculation. 1000x
A - Healthy B - Diseased
Note the nuclei filled with polyhedra (P) at different levels in the hypodermis. Many nuclei are seen liberating the polyhedra.

Fig. 35. Sections of hypodermis of healthy and NPV infected larvae of S.litura, 120 hr after inoculation. 1000x
A - Healthy B - Diseased
Note the dense groups of polyhedra (P).

Fig.34

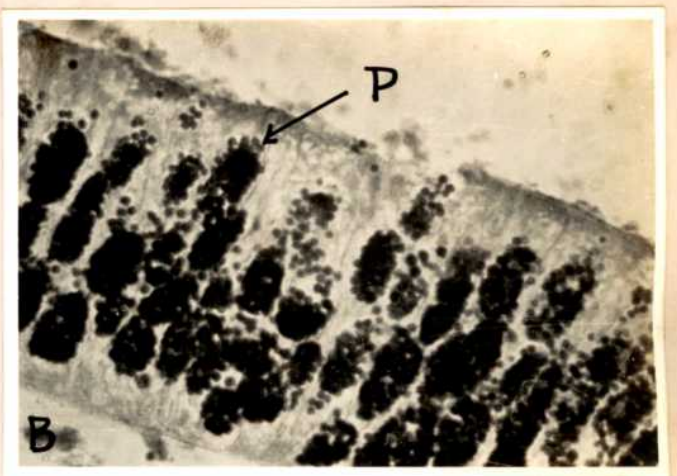
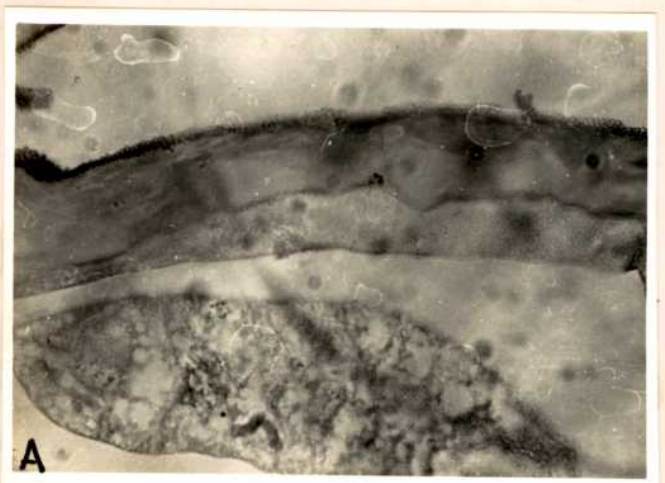


Fig.35



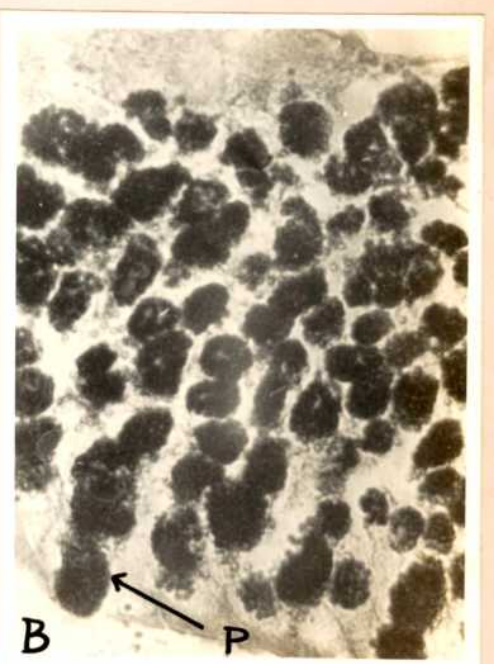


Fig.36, 37 and 38. Sections of trachea of NPV infected larvae of Spodoptera litura. 1000x

36. 73 hr after inoculation: Nuclei of tracheal matrix cells enlarged in size with fully developed polyhedra (P) inside them.

37. 96 hr after inoculation: Many nuclei ruptured liberating the polyhedra.

38. 120 hr after inoculation: Matrix cells in an advanced stage of infection.

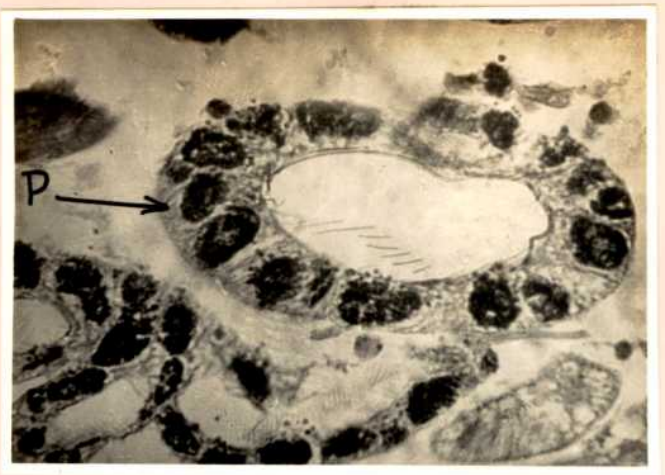


Fig.36

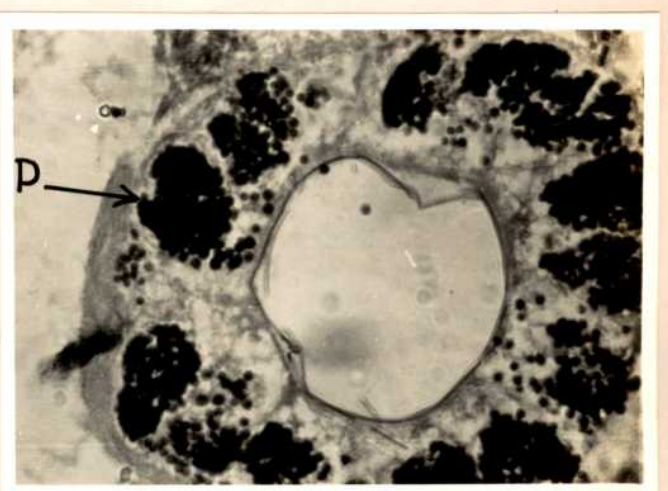


Fig.37

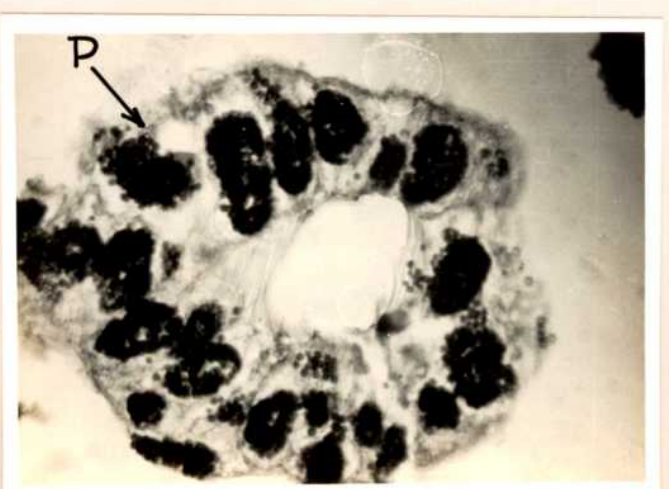


Fig.38

Fig. 39. Sections of fat body of healthy and NPV infected larvae of Spodoptera litura, 48 hr after inoculation. 1000x
A - Healthy B - Diseased
Note the hypertrophied nuclei (HN) and virogenic stroma (VS)

Fig. 40. Sections of fat body of healthy and NPV infected larvae of Spodoptera litura, 72 hr after inoculation.
A - Healthy, 1000x B - Diseased, 375x
C - Diseased, 1000x
Some nuclei in early stage (ES) of infection and some others with fully developed polyhedra (P).

Fig.39

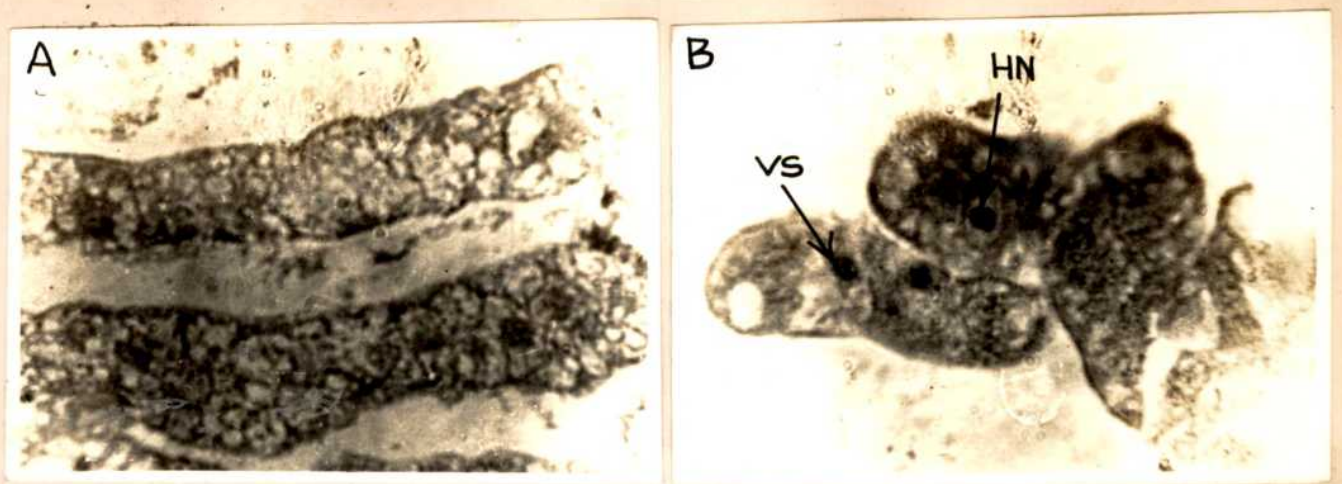
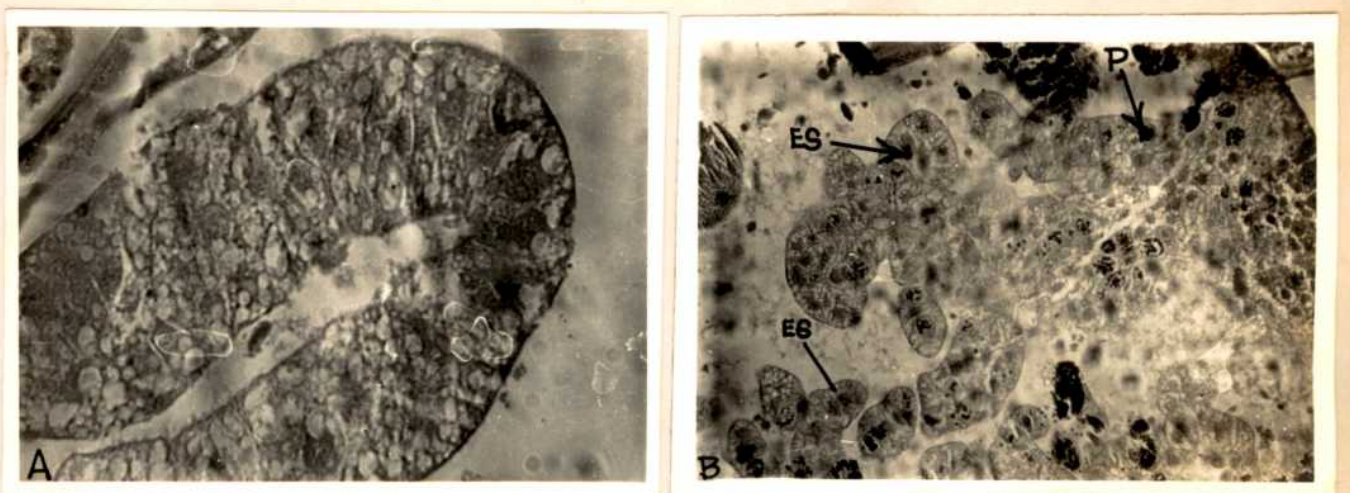


Fig.40



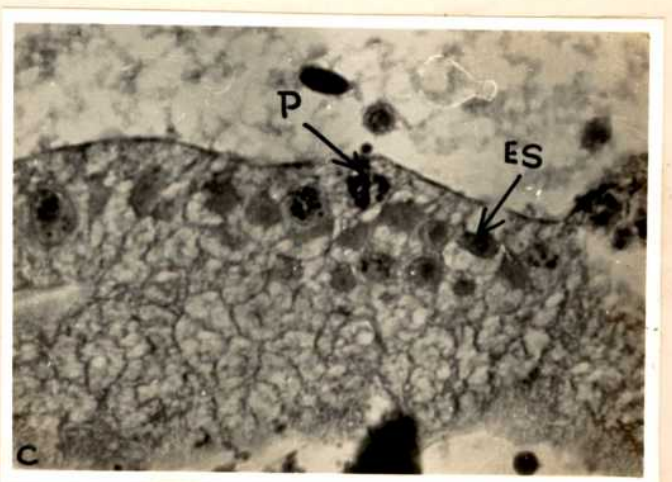
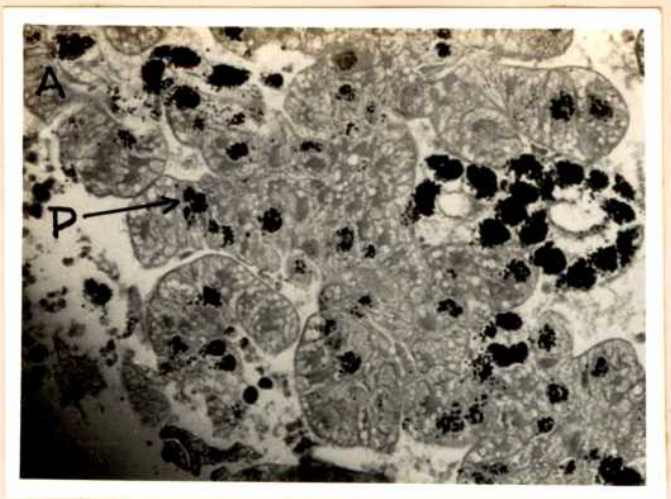
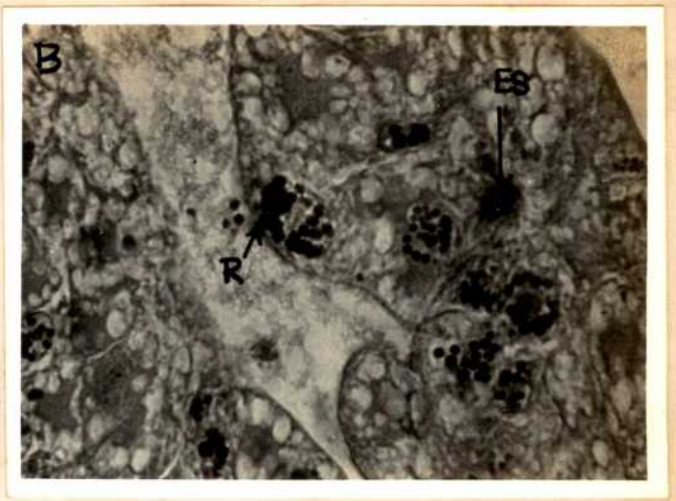


Fig. 41. Sections of fat body of healthy and NPV infected larvae of Spodoptera litura, 96 hr after inoculation.
A - Diseased, 375x B - Diseased, 1000x
Many nuclei filled with polyhedra (P) some nuclei in early stage (ES) of infection and a few nuclei ruptured (R).
C - Healthy, 1000x Note the large fat vacuoles (FV).

Fig. 42. Sections of fat body of healthy and NPV infected larvae of S.litura, 120 hr after inoculation, 1000x
A - Healthy: Large fat vacuoles (FV) in great numbers. B - Diseased: Polyhedra (P) present on all the nuclei. Note the absence of fat vacuoles.

Fig.41





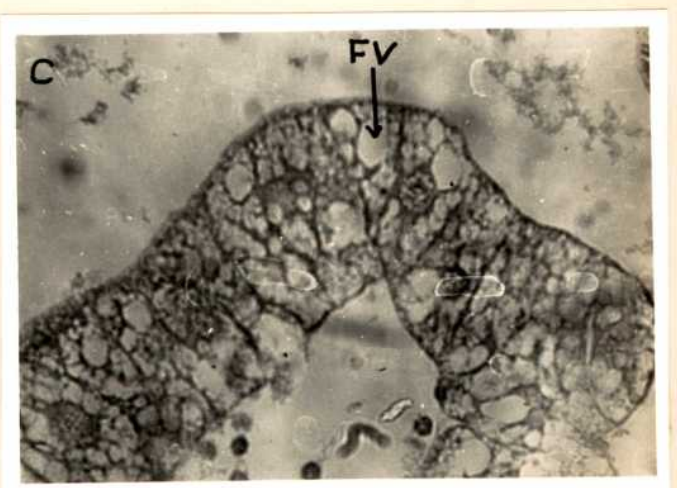
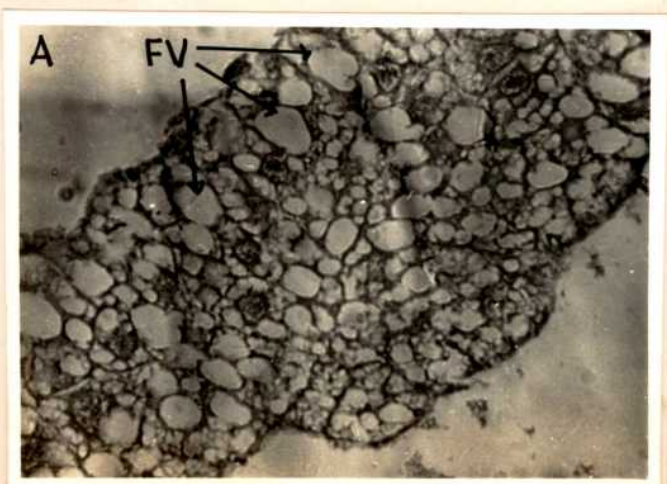


Fig.42



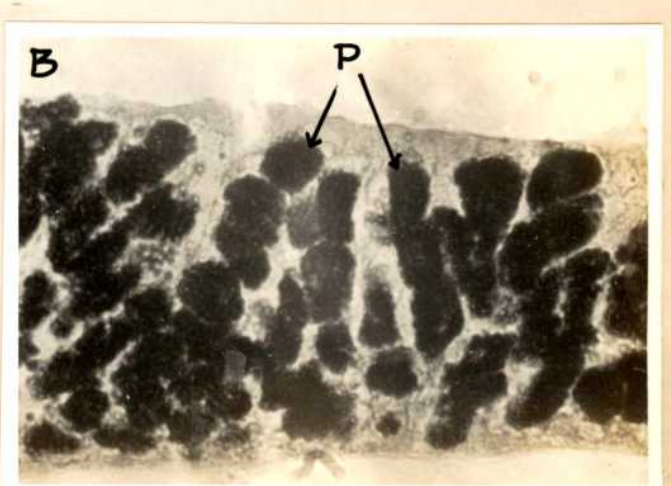


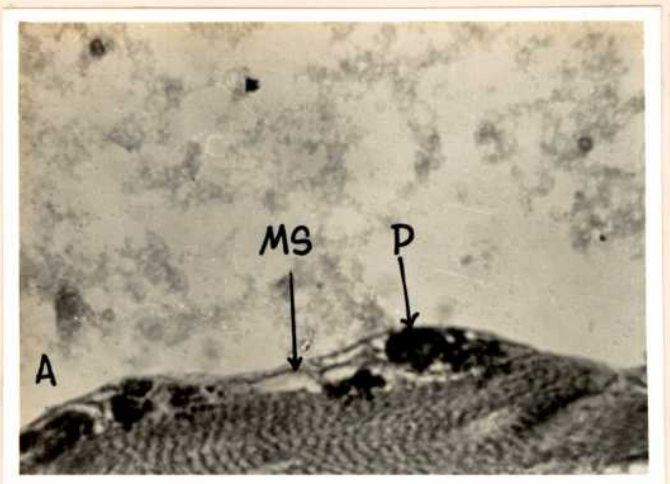
Fig. 43-45. NPV infected muscle tissues of Spodoptera litura, 1000x

43. 72 hr after inoculation:
A. Infection (P) of nuclei below the muscle sheath (MS).
B. Infected nuclei situated deep in the sarcoplasm.

44. 96 hr after inoculation

45. 120 hr after inoculation:
Note the dense groups of polyhedra below the sarcolemma and one cell lysing (L)

Fig.43



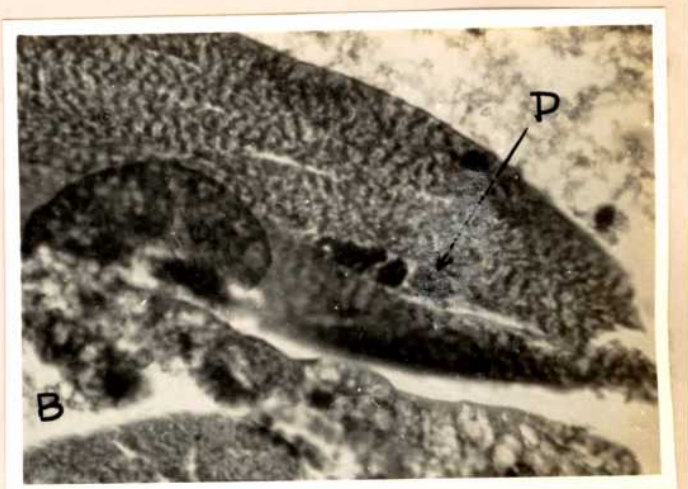


Fig.44

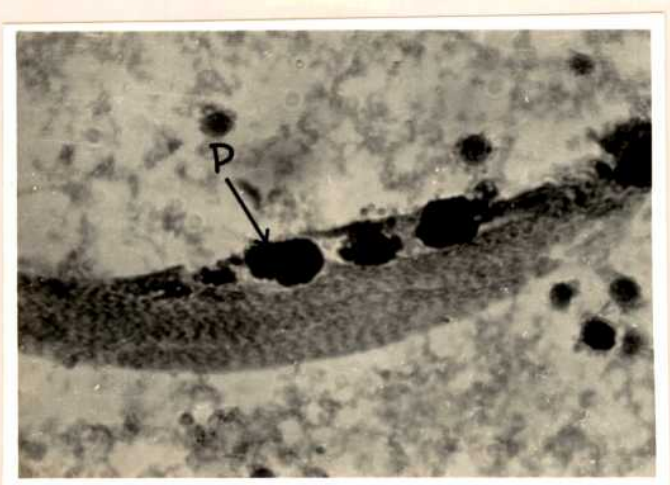


Fig.45

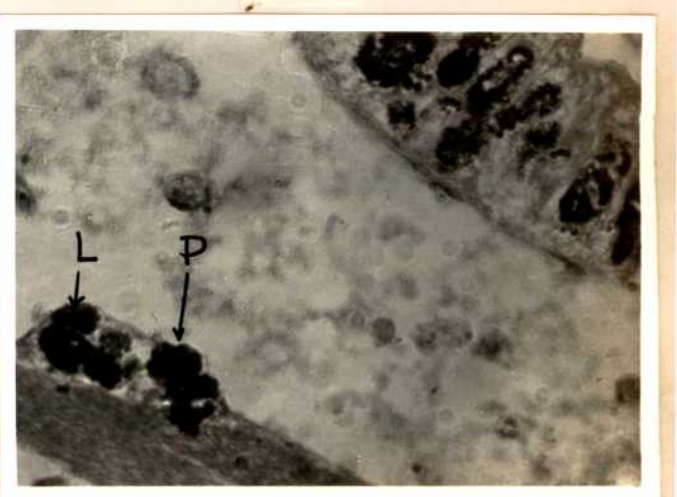


Fig. 46. Portion of a ventral nerve ganglion of NPV infected Spodoptera litura, 72 hr after inoculation, 1000x

Fig. 47. Section of brain showing polyhedra (P) near the periphery, 72 hr after inoculation, 375x.

Fig. 48. Infection of neurilemma (NL); 375x.
A - 72 hr after inoculation, 375x.
B - 96 hr after inoculation, 1000 x.

Fig.46

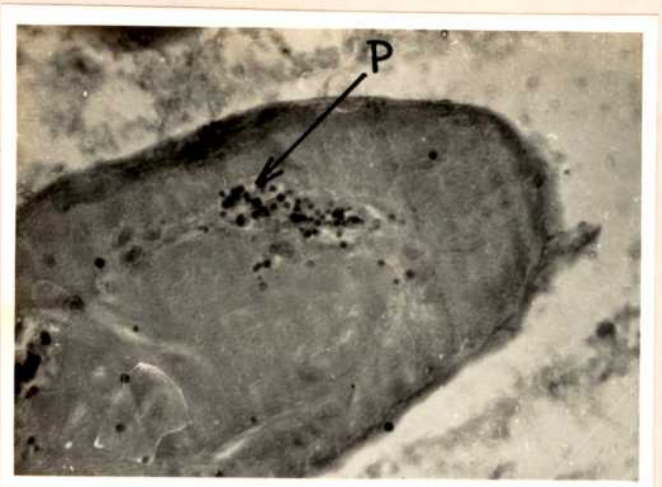


Fig.47

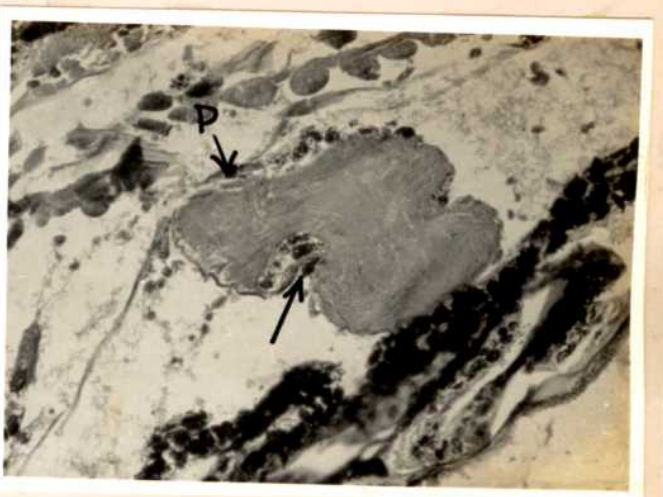
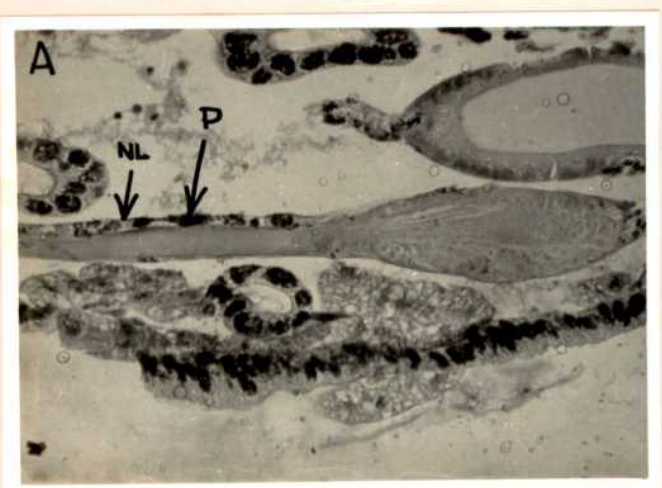


Fig.48



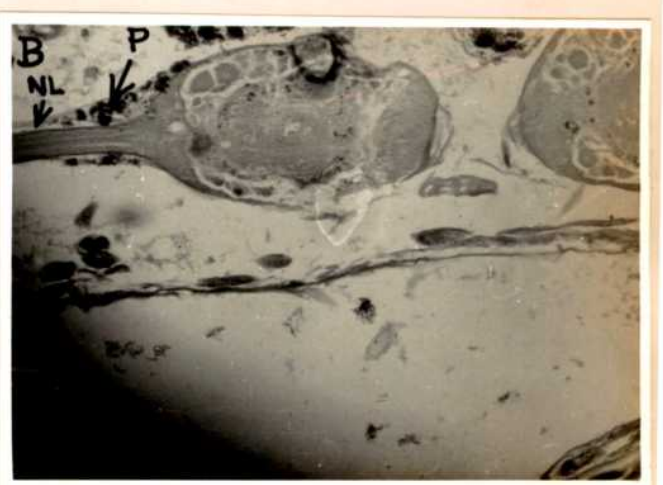


Fig. 49. Section of midgut (M) of S.litura showing infection of connective tissue (CT) surrounding it, 1000x

Fig. 50. Section of silk gland (SG) of S.litura showing infection of certain nuclei in the posterior region, 375x

Fig. 51. Section of wing bud (WB) of S.litura showing many nuclei with fully developed polyhedra (P). 375x

Fig. 52. A (375x) and B (1000x). Sections of alimentary canal showing polyhedra (P) in the nuclei of many cells at the region of cardiac valve (CV).

Fig.49

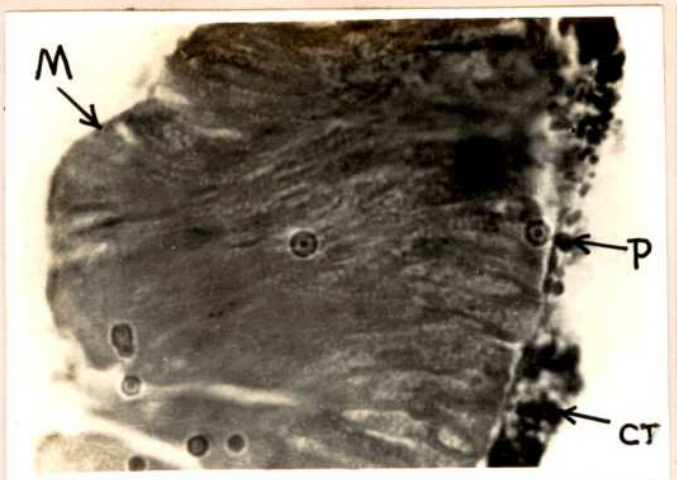


Fig.50

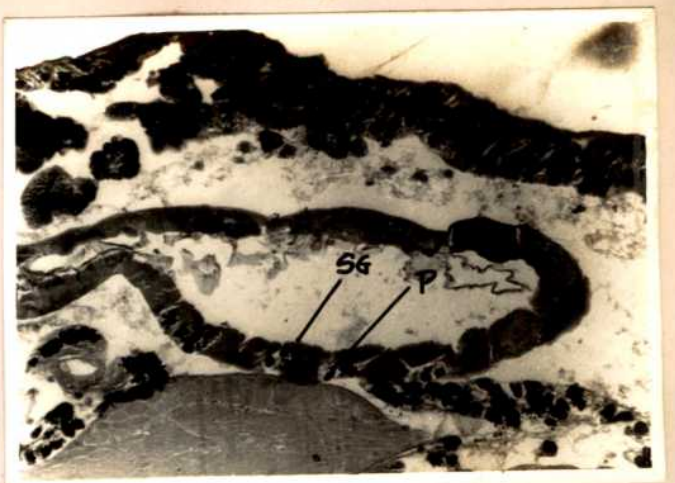


Fig.51

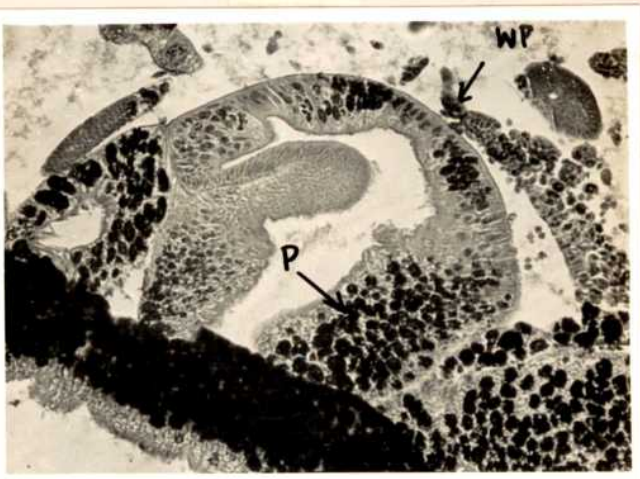
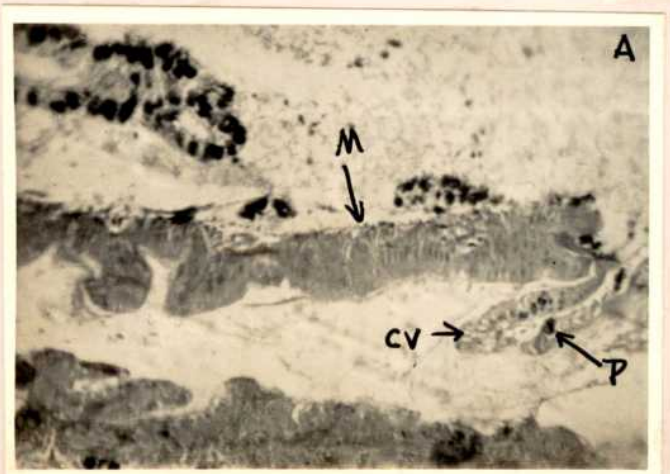
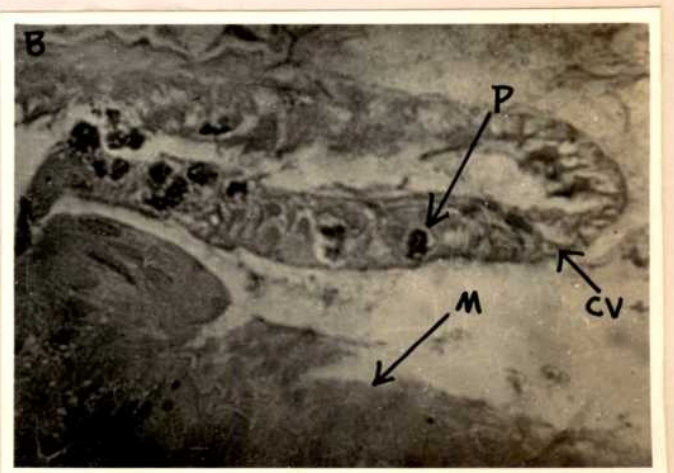


Fig.52





II

Fig. 53. Total nitrogen content in healthy and NPV infected larvae of S.litura at different intervals after inoculation

Fig. 54. The levels of uric acid in NPV infected as compared to healthy larvae of S.litura at different intervals after inoculation.

Fig.53

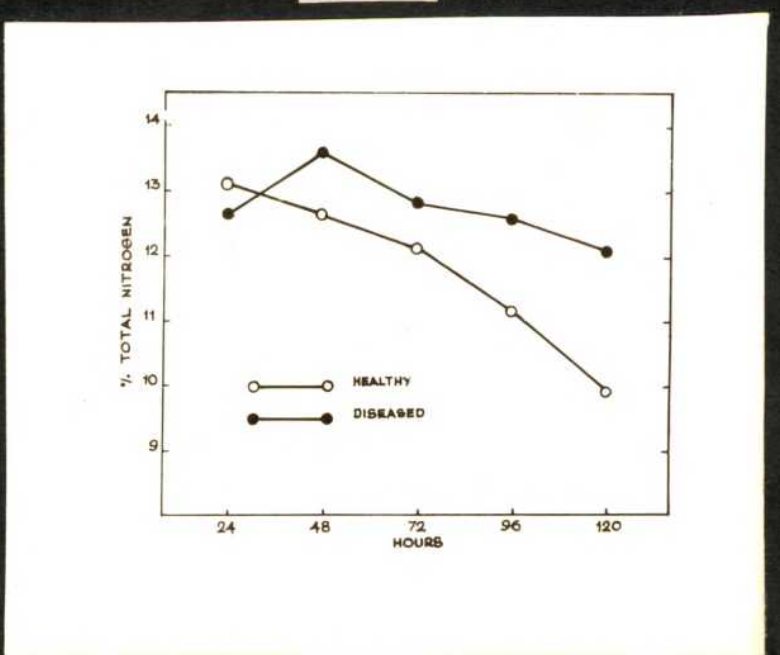

% TOTAL NITROGEN
14
13
12
11
10
9
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig.54

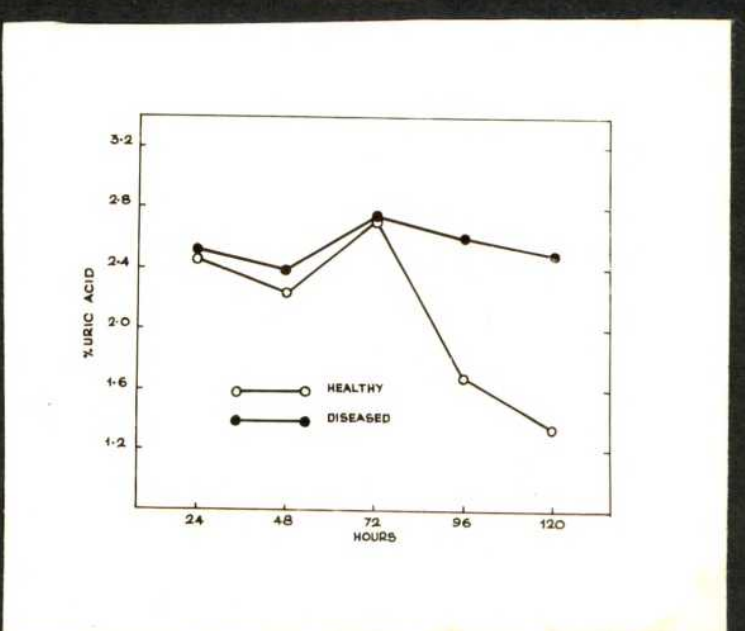

% URIC ACID
3·2
2·8
2·4
2·0
1·6
1·2
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig. 55. Protein content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig. 56. Total fat content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig.55

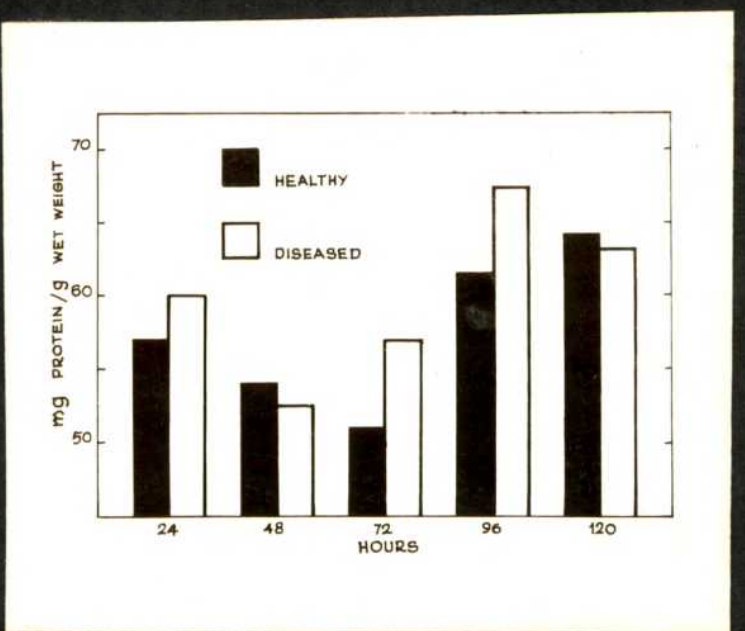

70
60
50
mg PROTEIN/g WET WEIGHT
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig.56

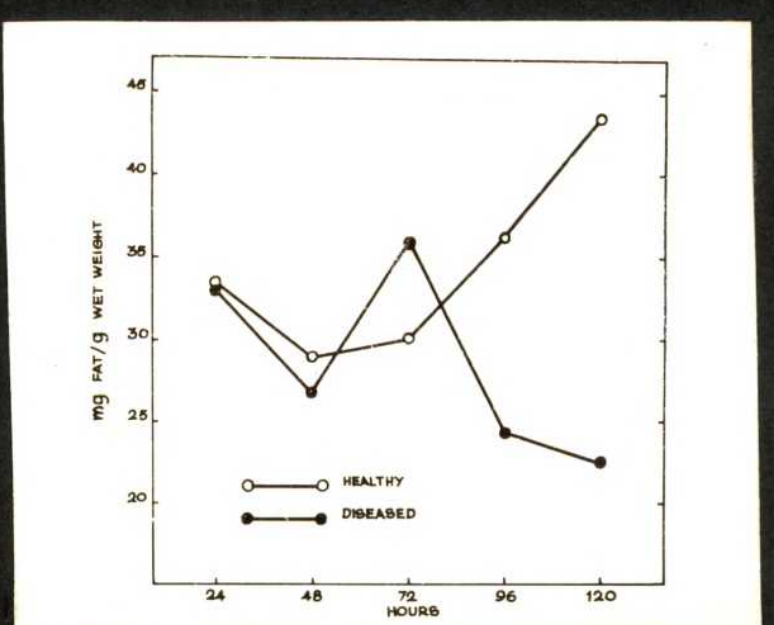

45
40
35
30
25
20
mg FAT/g WET WEIGHT
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig. 57. Total carbohydrates in healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig. 58. Glycogen content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig.57

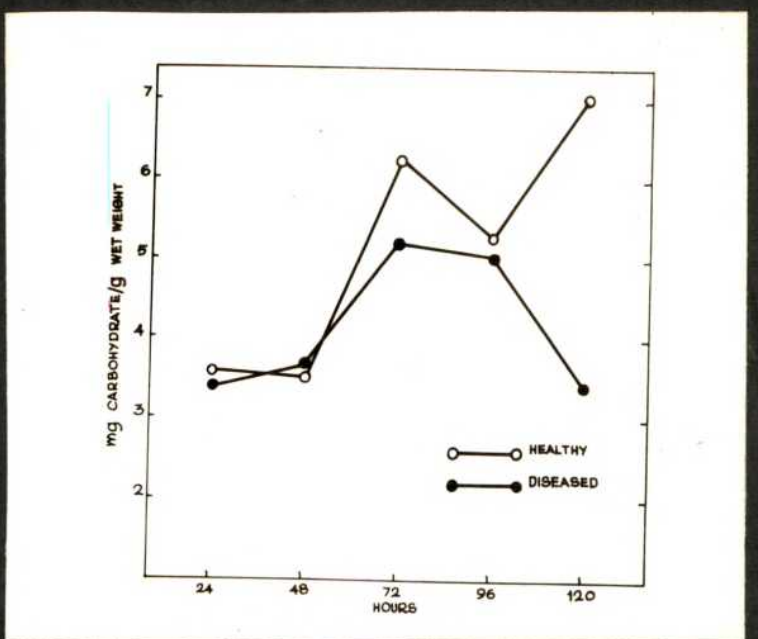

mg CARBOHYDRATE/g WET WEIGHT
7
6
5
4
3
2
24
48
72
96
120
HOURS
HEALTHY
DISEASED

Fig.58

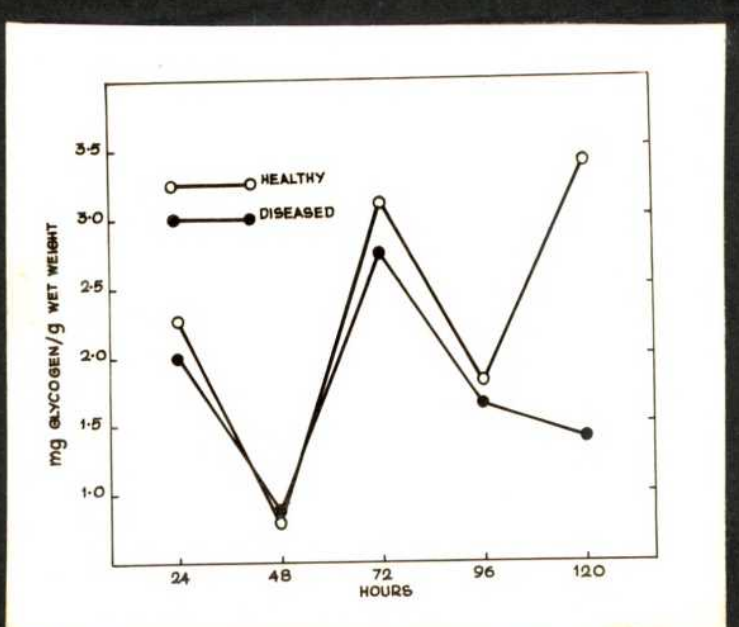

mg GLYCOGEN/g WET WEIGHT
3·5
3·0
2·5
2·0
1·5
1·0
24
48
72
96
120
HOURS
HEALTHY
DISEASED

Fig. 59. Changes in DNA content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig. 60. Changes in RNA content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig.59

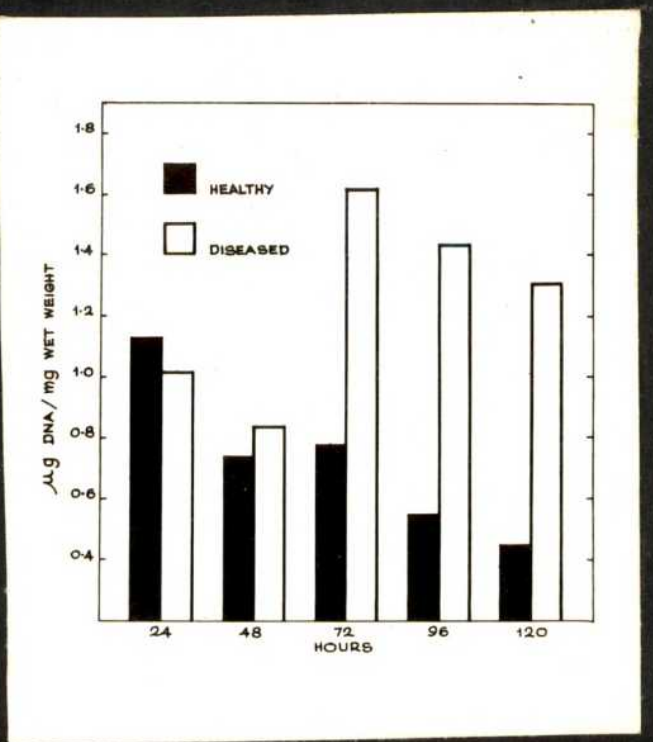

HEALTHY
DISEASED
µg DNA / mg WET WEIGHT
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
24
48
72
96
120
HOURS

Fig.60

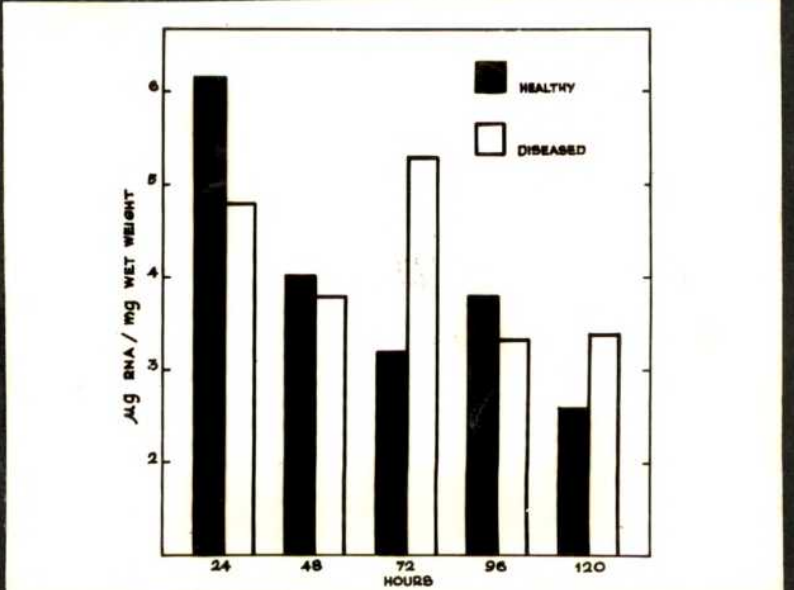

HEALTHY
DISEASED
µg DNA / mg WET WEIGHT
6
5
4
3
2
24
48
72
96
120
HOURS

Fig. 61. Changes in calcium content of healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig. 62. Magnesium content in healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig.61

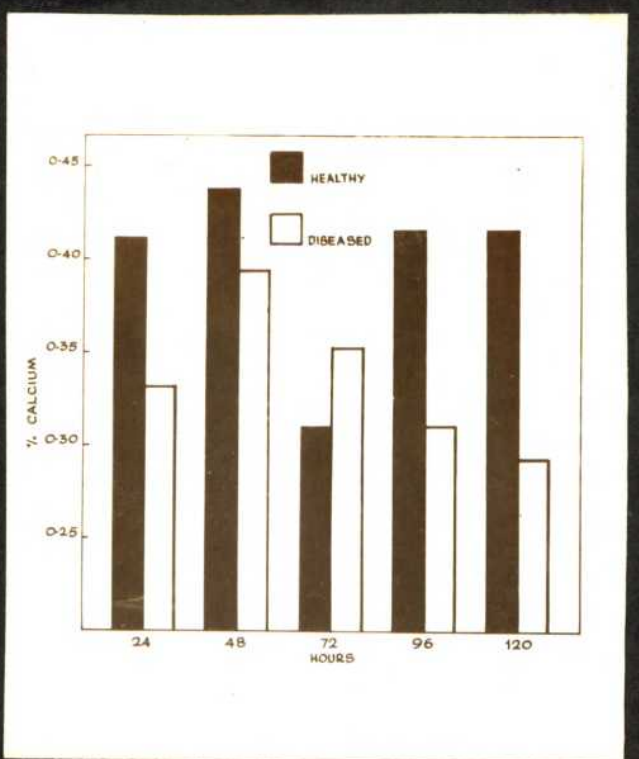

HEALTHY
DISEASED
% CALCIUM
0·45
0·40
0·35
0·30
0·25
24
48
72
96
120
HOURS

Fig.62

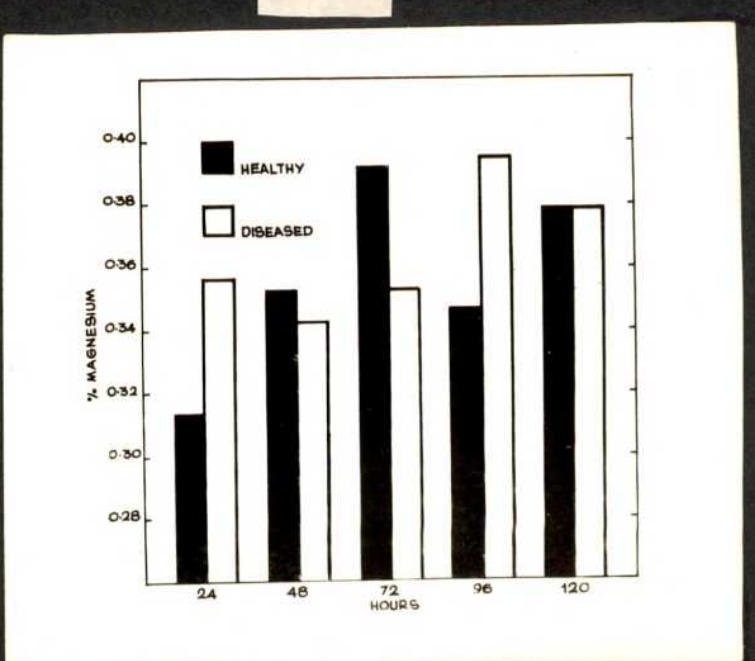

HEALTHY
DISEASED
% MAGNESIUM
0·40
0·38
0·36
0·34
0·32
0·30
0·28
24
48
72
96
120
HOURS

Fig. 63. Changes in sodium content of NPV infected larvae of S.litura as compared to normal larvae at different intervals after inoculation.

Fig. 64. Potassium content of healthy and NPV infected larvae of S.litura at different intervals after inoculation

Fig.63

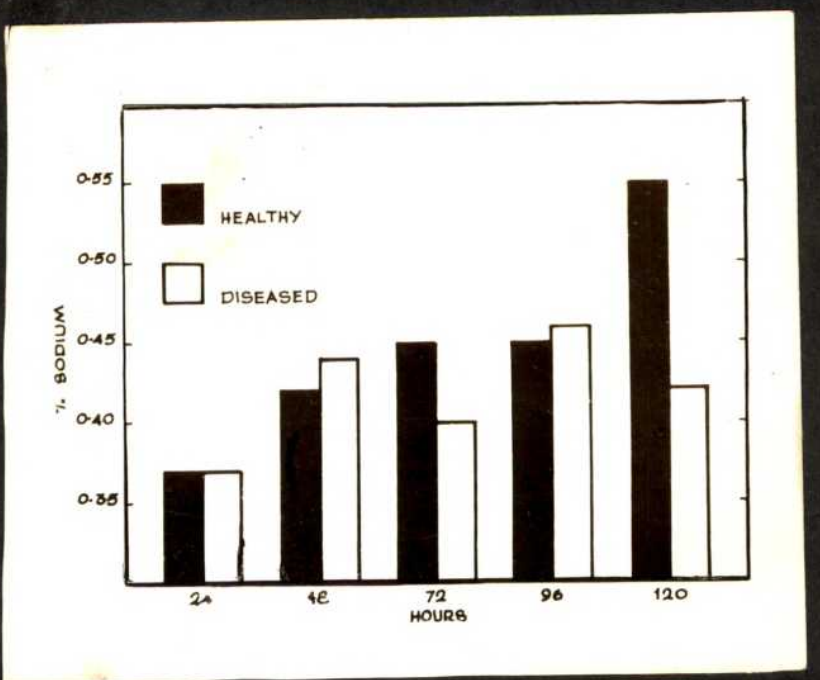

0·55
0·50
0·45
0·40
0·35
% SODIUM
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig.64

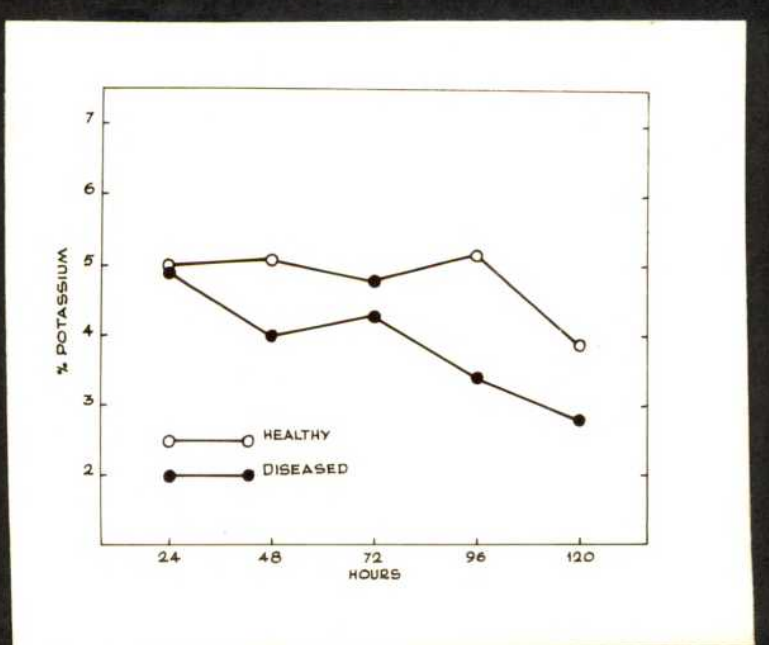

7
6
5
4
3
2
% POTASSIUM
HEALTHY
DISEASED
24
48
72
96
120
HOURS

Fig. 65. Levels of total phosphorus in NPV infected S.litura larvae as compared to healthy larvae at different intervals after inoculation.

Fig.65

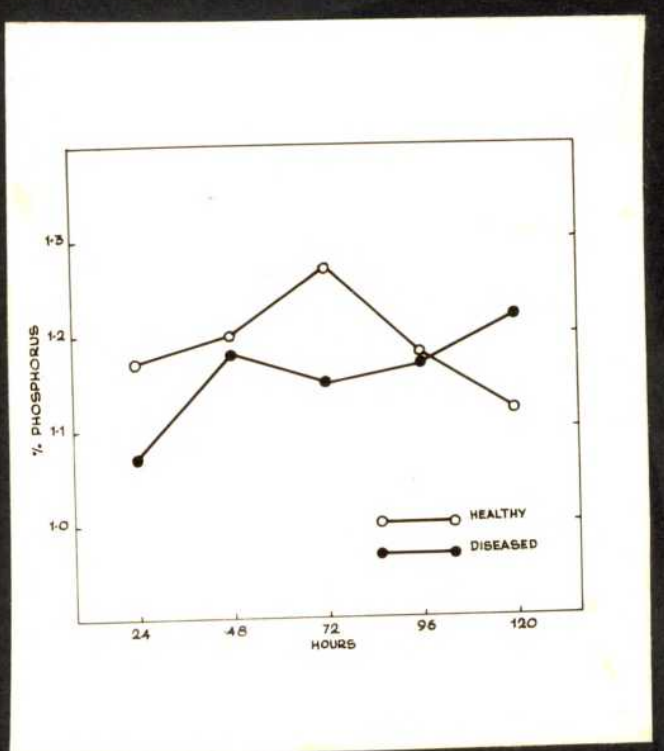
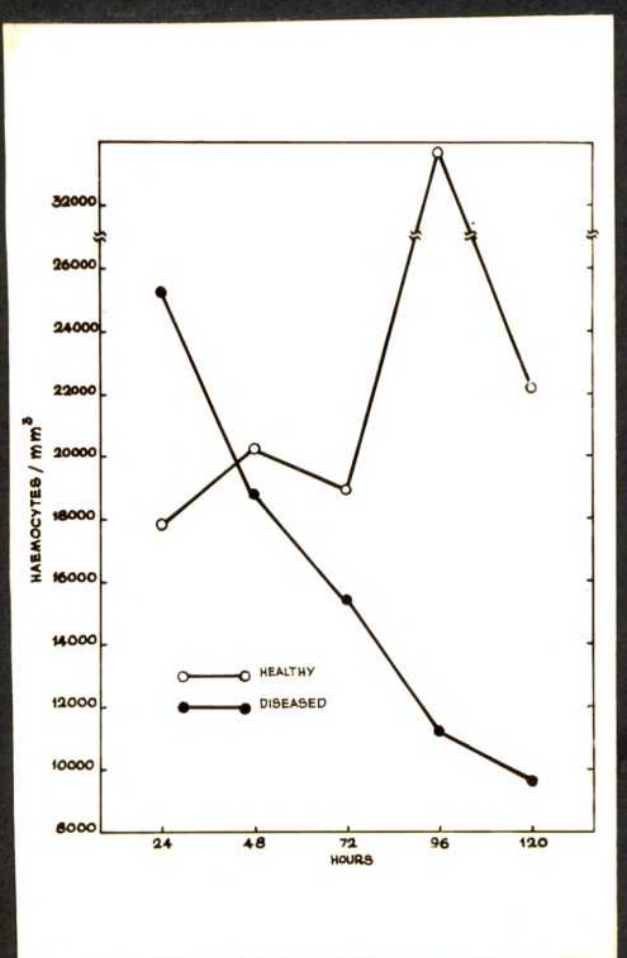


Fig. 66. Average number of circulating haemocytes in healthy and NPV infected larvae of S.litura at different intervals after inoculation.

Fig.66


32000
26000
24000
22000
20000
18000
16000
14000
12000
10000
6000
HAEMOCYTES / mm³
HEALTHY
DISEASED
24
48
72
96
120
HOURS

was very fragile and in most cases it ruptured liberating the now liquefied body contents (Fig.2). The cadavers darkened rapidly and dried up. The larvae generally died over the foliage or at the bottom of the container. The characteristic hanging down posture assumed by NPV infected larvae of many other Lepidoptera was not observed in this case.

Causative agent:

The polyhedra showed considerable variation in size and shape (Fig.3). Diameters of 160 polyhedra ranged from 0.7 to 1.7 μ with an average of 1.19 $\pm$ 0.015 μ (standard error of the mean). The rod shaped virus particles were arranged singly and in bundles of 2 to 5 rods (Fig.4). The carbon replicas showed no surface patterns (Fig.5).

NUCLEAR POLYHEDROSIS OF THE GRAM POD BORER, *Heliothis armigera* Hb.

A batch of larvae collected from redgram (*Cajanus cajan*) plants and reared in the laboratory in 1968 showed symptoms of a nuclear polyhedrosis. Haemolymph and tissue smears indicated the presence of polyhedra. Pathogenicity tests were made on fourth

instar larvae of H.armigera obtained from a disease free culture reared in the laboratory. Polyhedra collected from diseased larvae and purified by filtration and centrifugation were used for inoculation. A heavy suspension of polyhedra was mixed with redgram flour and fed to 100 test larvae kept individually in plastic containers. Approximately 500 mg of the wet mash was provided to each larva. One hundred larvae were kept individually in containers and provided with redgram flour wetted with sterile distilled water to serve as control. After 48 hr, fresh redgram seeds were provided as feeding material for both control and treated larvae. Observations were made on the symptoms and mortality. Out of the one hundred treated larvae, 98 died of nuclear polyhedrosis showing that the virus was highly infectious. None of the control larvae died of virus. The disease was later observed in the field also.

Signs and symptoms:

No symptoms were apparent until 3 to 4 days after ingestion of polyhedra. Thereafter the infected larvae exhibited loss of appetite and became sluggish. Feeding ceased almost immediately. During the initial stages, the skin took on an oily appearance. As the

disease progressed the cuticle became exceedingly fragile. In the later stages of the disease, the larvae sometimes discharged a greenish brown liquid from the mouth and a brownish fluid through the anus. Death occurred in 5 to 9 days with an average of 6 days. Caterpillars when infected in the earlier stages generally had a short incubation period. Dead larvae were found hanging down from the top of the containers (Fig.6) or other support to which they were attached by their posterior prolegs. They appeared as limp and flaccid sacs filled with the liquefied body contents. The cuticle ruptured at the slightest pressure, liberating the liquefied body contents. When dead the young larvae were yellowish or creamy and the older ones reddish or dark brown. The cadavers turned dark in a short time and dried up to a thin scale.

Larvae which ingested the polyhedra in the later stages pupated normally. But many of them died in the pupal stage. The infected pupae were observed to rupture easily on touch. The internal tissues had become disorganized and liquefied by this time.

Causative agent:

The polyhedra were irregular in shape (Fig.7) and showed considerable variation in size. Diameters of 302 polyhedra ranged from 0.5 to 1.4 μ with an average of 0.89 $\pm$ 0.012 μ (standard error of the mean). Sections of polyhedra showed virus rods occurring singly (Fig.8). Carbon replicas (Fig.9) did not show any surface patterns or structures.

Larvae showing atypical symptoms:

It could be observed on many occasions, that larvae inoculated with the virus died without showing the typical symptoms of nuclear polyhedrosis. Larvae inoculated in all the instars showed occasional cases of atypical death. Feeding by such larvae ceased in a late stage and the larvae remained alive for a fairly long period extending to 10 to 12 days. During this period the larvae were quite sluggish. But no colour change or other signs were apparent. At death the cuticle did not rupture. The internal tissues were in a partially disorganized state, but not liquefied. Haemolymph and tissue smears however, showed presence of polyhedra. Further it was also observed that large numbers of blood cells were infected.

their moult to the particular instar. Fifty larvae of each instar were fed a dosage of 10^6 polyhedra per larva as outlined under "Materials and Methods". Another set of 50 larvae of each instar treated similarly but without the virus inoculum served as control.

The experiments were conducted at room temperature which varied between 22.3° and 29.6°C. The relative humidity during the period ranged from 70 to 97 per cent with a mean of 87. Diagnosis of the dead larvae was done by microscopic examination of squashed preparations or tissue smears stained with giemsa.

The incubation period and percentage mortality of the different instars of the larvae are presented in Table 1.

It is evident from the table that the mean incubation period increased as the instar of the larvae advanced. Thus from 4 days for the second instar larvae the incubation period was raised to 7.7 days for the sixth instar larvae. Further the second, third and fourth instar larvae were highly susceptible to the virus recording a mortality of 96 to 100 per cent. The fifth instar larvae were less susceptible to the virus,

to early fourth instar larvae as described under "Materials and Methods". Fifty larvae were tested for each dose. A set of 50 larvae treated similarly but without the infective material served as control.

The experiments were conducted at room temperature which varied between 22.3° and 29.6°C. The relative humidity during the period ranged from 70 to 97 per cent with a mean of 87. Diagnosis of dead larvae was done as described under the previous experiment.

The percentage mortality and incubation period of the larvae when inoculated with the different dosages of polyhedra are presented in Table 2.

TABLE 2

Per cent mortality and incubation periods of fourth instar larvae of S.litura when fed with different dosages of nuclear polyhedra

Number of polyhedra/ larva	Incubation period (days)		Number dead		% mortality due to virus
	Range	Mean	Polyhedrosis	Other causes	
10^3	6-9	6.5	22	2	44
10^4	6-8	6.3	33	-	66
10^5	5-7	5.8	50	-	100
10^6	4-6	5.3	50	-	100
Control	-	-	-	-	-

It shows that the mortality increased with the dosage recording 100 per cent kill at dosages of 10^5 and 10^6 polyhedra per larva. Further the average incubation period decreased as the dosage was increased. Thus the average incubation period was 6.5 days with the lowest dosage and 5.3 days when the highest dosage was applied.

The causative agent:

The polyhedra (Fig.19) were irregular in shape and varied considerably in size. The diameters of 87 polyhedra ranged from 1.2 to 2.8 μ and averaged 1.86 $\pm$ 0.025 μ (standard error of the mean). Sections of polyhedra (Fig.20) showed virus rods occurring singly and in bundles of 2 to 14 rods. Carbon replicas of the polyhedra (Fig.21) did not show any surface pattern.

Effect of the disease on the size and weight of the larvae

a) Wet and dry weights:

These studies were made on fourth instar larvae inoculated with 10^5 polyhedra per larva as outlined under "Materials and Methods". Thirty larvae each were selected at random from both treated and untreated groups at 24 hr intervals for a period of 120 hr. The first

sampling was done 24 hr after inoculation. The wet weight was determined after dividing the larvae into 3 groups of 10 each in weighing bottles. Then each group was dried to constant weight at 100°C in a hot air oven. The mean wet and dry weights per larva were calculated and expressed as mg per larva. The moisture percentage was also worked out.

The results are presented in Table 3 and illustrated in Figs. 22 and 23. It may be seen that the

TABLE 3

Changes in wet and dry weights and moisture content of healthy and diseased caterpillars of *S.litura*

Post inoculation period in hr	Wet weight/larva - Mean (mg)		Dry weight/larva - Mean (mg)		Moisture percentage	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
24	49.46	43.89	5.98	6.01	87.86	86.80
48	127.45	123.83	16.93	17.21	86.70	86.30
72	234.77	254.51	28.95	33.45	87.67	86.10
96	584.83	360.70	76.10	50.08	86.97	86.30
120	997.69	326.77	151.03	51.08	84.80	84.37

CD (P = 0.05): Comparison of wet weights

Healthy VS Diseased = 24.88
Periods = 39.33
Interaction = 55.63

healthy larvae increased in wet weight with advancing age, the mean weight at 120 hr showing a twenty fold increase over that at 24 hr. The diseased larvae also gained in weight until 72 hr after treatment. At 96 hr also it recorded a slight increase, less than in healthy larvae. After 96 hr post-treatment the diseased larvae did not increase in weight, but recorded a slight decrease which was not significant statistically. Thus, the results show that a retardation of growth occurred in diseased larvae from 72 hr after treatment. A comparison between the wet weights of healthy and diseased larvae at different periods would show that the diseased larvae had a slightly higher weight at 72 hr. But at 96 and 120 hr post-treatment the diseased larvae had considerably low weights, the percentage of reduction from the weight or healthy larvae being 38.32 and 67.24 respectively.

The changes in dry weight of both healthy and diseased larvae followed a pattern similar to their corresponding wet weight. But the dry weight of diseased larvae remained more or less constant at 96 and 120 hr after treatment. There was no marked difference between the moisture contents of healthy and diseased larvae, though it always recorded a slightly increased

level in healthy larvae. At 120 hr, both healthy and diseased larvae showed a decrease of about 2 per cent over the moisture content at 96 hr.

b) Length of the larvae:

The length of larvae was determined at 24 hr intervals for a period of 120 hr after inoculating fourth instar larvae with 10^5 polyhedra/larva. The first measurement was made 24 hr after treatment. Twenty five larvae each in control and treated group were kept in individual labelled containers and the length of each determined daily. A plastic metric scale was used for the measurement. The larva to be measured was slightly pressed to the scale and the reading taken. The average length of the larva in mm was calculated for each interval for both treated and control group. The statistical 't' analysis was done for comparing the difference between means.

The data on the mean length of healthy and diseased larvae are presented in Table 4 and illustrated in Fig.24. It ~~is~~ will be observed that the length of healthy larvae increased steadily with advancing age. Thus from 14.56 mm observed at 24 hr the length of healthy larvae increased to 37.48 mm at 120 hr. The diseased

TABLE 4

Mean length of healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Mean length of larva in mm ± S.E.	
	Healthy	Diseased
24	14.56 ± 0.63	14.12 ± 0.14
48	18.60 ± 0.33	17.92 ± 0.26
72	24.04 ± 0.30	25.72 ± 0.33
96	30.48 ± 0.60	25.76 ± 0.54
120	37.48 ± 1.18	25.64 ± 0.59

larvae also showed a steady increase until 72 hr after treatment. But after that no marked changes were noted, the larvae showing the same length at 72, 96 and 120 hr.

Comparing the length of both healthy and diseased larvae, it is seen that there was no significant difference in the length at 24 and 48 hr after treatment. At 72 hr, the diseased larvae were longer than corresponding healthy ones by 1.68 mm. The statistical 't' analysis showed this difference as significant. At 96 and 120 hr the diseased larvae were significantly shorter than comparable healthy ones.

Effect of the disease on the moulting of the larvae:

The effect of virus infection on the moulting was studied by inoculating fourth instar larvae within 6 to 8 hours of their moult. Two concentrations viz., 10^3 and 10^5 polyhedra per larva were fed to 50 larvae each as outlined under "Materials and Methods". Fifty larvae treated similarly but without the infective material served as control.

The experiments were conducted at room temperature which varied between 22.1° and 30.2°C. The mean relative humidity during the period was 92 per cent with a range from 74 to 98.

Table 5 summarizes the results of observations on the effect of virus infection on moulting. The healthy larvae underwent two moultings (4th and 5th), the first within 2 days and the other within 4 days. Among the larvae inoculated with the 10^3 polyhedra, all had the first moult (4th), but only 29 underwent the second moulting (5th). The remaining 21 larvae which were infected did not have the second moult (5th). Out of the fifty larvae inoculated with 10^5 polyhedra all had the first moulting (4th) while only one underwent the second moulting (5th). This larvae escaped infection

TABLE 5

Effect of NPV infection on the moulting of S.litura when infected in the fourth instar

Dosage of polyhedra/larva	Incubation period	No. of larvae moulted to		Number of larvae dead due to	
		5th instar	6th instar	Polyhedrosis	Others
10^3	6-9	50	29	21	-
10^5	5-7	50	1	49	-
Control	-	50	50	-	-

and had pupated. The results thus show that none of the infected larvae, had undergone the second moult following inoculation and this moult generally occurred in healthy larvae, within 4 days after the initiation of the test.

Effect of the disease on the food consumption of the larvae:

Observations were made on the quantity (area) of castor leaf eaten by the healthy and diseased larvae. Twenty five early fourth instar larvae were inoculated with a dosage of 10^5 polyhedra per larva as outlined under "Materials and Methods". A similar number of larvae of the same age were kept as control. Each

larva was kept individually in a battery jar. To measure the area of leaf consumed by the larvae each larva was provided with a single whole leaf of castor. Before being fed to the larva the original contour of the leaf was marked on a metric graph paper. The petiole of each leaf was kept dipped in water contained in a specimen tube. After 24 hr of feeding, the larvae were tranferred to fresh leaves. The area fed by each larva was measured with the help of the graph paper on which the outlines had been marked. The area of consumption was estimated as square mm. Estimations were done in this way for 120 hr at 24 hr intervals. The statistical 't' analysis was used for comparing the difference between means.

The results are summarized in Table 6 and illustrated in Fig.25. There was no significant difference in the leaf area consumed during the first day by healthy and diseased larvae. On the second day, the diseased larvae consumed a significantly larger area, showing an increase of 105.08 per cent over healthy. The leaf area fed by both healthy and diseased larvae were again equal during the third day. But the diseased larvae consumed a significantly lesser area of leaf on the fourth day the reduction from that of healthy being

TABLE 6

Area of castor leaf consumed by healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Leaf area consumed/larva in mm^2 (Mean) ± S.E.		
	Healthy	Diseased	% increase (+) or decrease(-) over healthy
24	194.20 ± 15.47	297.00 ± 21.89	+ 0.95
48	344.00 ± 25.95	705.50 ± 83.01	+105.08
72	1118.10 ± 438.02	1122.75 ± 254.26	+ 0.42
96	2055.10 ± 32.58	885.50 ± 401.74	- 45.36
120	5314.75 ± 313.52	---	---

45.36 per cent. The diseased larvae did not feed on the fifth day. The results thus show that the disease caused a retardation of feeding on the fourth day and feeding was completely inhibited on the fifth day.

HISTOPATHOLOGY

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

24 hr after inoculation:

No changes were visible in any tissue.

48 hr after inoculation:

Most of the cells of the hypodermis and some cells of the fat bodies showed early signs of infection.

72 hr after inoculation:

Polyhedra were distinctly visible in most of the cells of hypodermis, tracheal matrix and few cells of fat bodies (Fig.26). More cells of the fat bodies showed early signs of infection. Many blood cells also had polyhedra in their nuclei. Polyhedra were also visible in muscle cells, nerve ganglia, neurilemma of nerva cord, brain, salivary gland and connective tissue surrounding the midgut.

96 hr after inoculation:

The infection had fairly advanced in hypodermis and tracheal matrix. Infection of fat body spread to more nuclei and polyhedra were visible in them (Fig.27). Many blood cells also showed advanced stage of infection. Some cells of the foregut and most cells of the wing bud had shown polyhedra in their nuclei by this time.

120 hr after inoculation:

The hypodermis and tracheal matrix showed signs of disintegration. Infection appeared more or less completely over the fat body. Large number of blood cells were seen heavily loaded with polyhedra and some of them had even ruptured releasing the polyhedra. Infection spread completely over the cells of the wing buds. Some of the muscle cells were also in an advanced stage of infection (Figs.28, 29, 30, 31).

The precise histopathological changes observed in the different tissues due to the virus infection are detailed below:

Hypodermis:

Photomicrographs of the changes occurring in the infected hypodermis as compared to non-infected tissue are given in Figs.32, 33, 34 and 35. The average thickness of the hypodermal layer as measured at random in 50 different regions/day of infection are furnished in Table 7.

The normal hypodermis consisted of a single layer of cells, resting on a basement membrane. The cells were usually cuboidal in shape, with their nuclei

TABLE 7

Thickness of hypodermis of healthy and diseased larvae of Spodoptera litura at different periods after inoculation

Post inoculation period in hour	Average thickness in μ ± S.E.	
	Healthy	Diseased
24	11 ± 0.99	13 ± 0.69
48	20 ± 1.61	34 ± 1.27
72	16 ± 5.38	96 ± 4.17
96	17 ± 1.29	121 ± 3.82
120	16 ± 1.07	121 ± 3.82

situated more towards the basement membrane. No apparent changes were noticeable with light microscope at 24 hr post-inoculation. The average thickness of both healthy and diseased hypodermis did not show any marked variation. Early signs of infection were observed in the hypodermis of larvae fixed 48 hr after inoculation. At this stage (Fig.32) most of the nuclei were hypertrophied and occupied a more central position compared to the normal cell nuclei. Many of the hypertrophied nuclei showed chromatin condensation to a unified mass at the center (Virogenic stroma of Xeros,

1956) with clear "ring zones" around the peripheral area. Some of the cells had hypertrophied nuclei but no virogenic stroma. The whole hypodermal layer itself appeared slightly swollen compared to the normal tissue.

In larvae fixed 72 hr after ingestion of the polyhedra, the hypodermal cells in most regions of the body showed their hypertrophied nuclei filled with fully developed polyhedra (Fig.33). Polyhedra were either few or not visible in cells of the anterior region of the thorax and lower region of the head. The hypodermal layer appeared considerably swollen in most regions. On an average the infected tissue measured 96 μ in thickness with a range of 44 to 160 μ whereas the normal layer varied in thickness from 8 to 28 μ with an average of 16 μ. The thickening of the layer was not so much marked in the anterior region of the thorax and venter of the head.

By 96 hr post-treatment (Fig.34) the hypodermis was enormously swollen recording nearly seven fold increase in thickness over the uninfected layer. The average thickness of the infected layer was 121 μ with a range of 52 to 192 μ. The normal layer on the other

hand, measured only 17 μ in thickness with a range of 8 to 28 μ. The cells were highly elongated at this time and the location of nuclei in different levels in the hypodermal layer with polyhedra gave it a stratified appearance. Many nuclei had ruptured and liberated the polyhedra by this time. The cells of the hypodermal layer in the anterior region of the thorax and lower region of the head also had fully developed polyhedra in their nuclei. But the stratified appearance observed in the other regions were absent here.

In larvae fixed 120 hr after inoculation, the hypodermis had more or less completely retracted from the cuticle in many regions and signs of disintegration were visible with the constituent cells getting separated from each other (Fig.35). Polyhedra were seen liberated from many nuclei. Measurement of the thickness of the hypodermis showed no conspicuous change from that of 96 hr.

Tracheal matrix:

At 24 and 48 hr following inoculation no well defined indications of infection could be observed in tracheal matrix cells. In larvae fixed 72 hr post-

treatment nuclei of tracheal matrix cells (Fig.36) throughout the body appeared conspicuously enlarged in size and contained fully developed polyhedra. The nuclei enlarged further as the polyhedra increased in number and at 96 hr post-treatment many nuclei (Fig.37) even ruptured liberating the polyhedra into the surrounding tissues and haemocoel. By 120 hr post-inoculation, the matrix cells had started disintegration and they had lost their normal structure in many spots showing disorganized cell structure (Fig.38).

Fat body:

The changes observed in the adipose tissue during the course of NPV infection as compared to those in the healthy larvae are illustrated in Figs.39, 40, 41 and 42. Table 8 shows size change as determined by measurement of 50 nuclei/day of infection.

There was no apparent difference between healthy and infected tissue for the first 24 hr following infection. The normal fat body cells were characterised by the presence of a large number of fat vacuoles in their cytoplasm.

TABLE 8

Changes in diameter of NPV infected and healthy fat body nuclei of the larvae of S.litura

Post-inoculation period in hour	Mean diameter in μ ± S.E.	
	Healthy	Diseased
24	8.7 ± 0.47	8.9 ± 0.42
48	8.0 ± 0.40	10.7 ± 0.41
72	10.5 ± 0.39	15.6 ± 0.44
96	10.1 ± 0.58	20.2 ± 1.99
120	10.0 ± 0.28	22.9 ± 1.18

By 48 hr post-inoculation some of the nuclei were hypertrophied and in some of these chromatin appeared to have condensed to a central mass (Virogenic stroma of Xeros, 1956) with "ring zones" around (Fig.39). Fat vacuoles were smaller and less numerous in the infected cells when compared with uninfected cells. But for this change, the adipose tissue looked alike in infected and healthy larvae.

In larvae fixed 72 hr after inoculation nuclei at various stages of infection could be seen in different

lobes of the adipose tissue (Fig.40). Even adjacent cells could be found to be at different stages of infection. By this time the number of hypertrophied nuclei increased considerably. The average diameter of the fat body nuclei in the infected larvae showed a marked increase. Many nuclei had fully developed polyhedra inside. Some of the nuclei showed small polyhedra in the ring zone while a few others had both small and fully formed polyhedra. The fat vacuoles were smaller in number and size in the infected areas. The normal fat body, on the other hand, had very large fat vacuoles in great numbers.

By 96 hr after inoculation, the infection had spread to more nuclei and fully formed polyhedra were present in large numbers of nuclei (Fig.41). The size of nuclei of infected fat body showed a two fold increase as evidenced by the diameter measurements. At this stage also the lack of uniformity of infection in the fat body was evident. While many nuclei were in the initial stage of infection it was interesting to note that some nuclei had ruptured and released the polyhedra. The fat vacuoles showed a marked reduction in size and number.

In larvae fixed 120 hr after inoculation the infection had spread more or less completely over the adipose tissue (Fig.42). The average size of the fat body nuclei in the infected larvae still continued to increase. Fat vacuole had almost disappeared from most areas. The healthy larvae on the other hand had fat bodies with large fat vacuoles and small nuclei. In the infected tissues a majority of cells had fully formed polyhedra inside their hypertrophied nuclei. Disintegration of the tissue was visible in many lobes. However, it was not difficult to recognize even at this stage nuclei which were in an early period of polyhedral formation.

Blood cells:

Phase contrast examination of haemolymph from heat fixed larvae showed the presence of polyhedra in the nuclei of many cells at 72 hr post-inoculation. By 96 hr more cells were seen infected. Some of the cells had ruptured liberating the polyhedra into the haemolymph. The infection further advanced by 120 hr post-inoculation when large number of nuclei fully laden with polyhedra, were visible. No attempt was made to identify the types of haemocytes infected.

Muscle tissue:

Infection of muscles were observed from 3 days after inoculation. At this stage polyhedra could be seen in the nuclei disposed immediately beneath the muscle sheath (Fig.43 A), as well as those placed deep in the sarcoplasm between the fibrillae (Fig.43 B). As a result of infection and hypertrophy of the nuclei, in many cases, sarcoplasm showed an apparent vacuolation and the muscle sheath appeared to have loosened in the vicinity of infected nuclei. The infection advanced further by 96 and 120 hr post-inoculation (Figs.44, 45). Though muscles of all regions of body were infected, the infection was generally limited to few spots in a fibre only. Rarely large number of nuclei of a single fibre were also seen infected.

Nervous system

Polyhedral formation was observed in the brain, ventral nerve ganglia and the neurilemma surrounding the nerve cord. Polyhedra were visible in all the above mentioned tissues from 72 hr after inoculation. In the ganglia (Fig.46) polyhedra were observed in the neuropile mass. Infection of brain was noted in certain foci, near the periphery (Fig.47). However, the infection

of brain and ganglia were of low magnitude only. But the infection appeared severe in the neurilemma (Fig.48 A, B) of nerve cord. The neurilemma appeared loosened from the inner cord as a result of the infection.

Connective tissue surrounding the midgut

Polyhedra were observed at 72 hr after inoculation in several spots in the connective tissue surrounding the midgut (Fig.49). The infection appeared to be of the matrix cells of tracheae associated with the connective tissue.

Silk glands

In larvae fixed 72 hr post-inoculation, some cells of the posterior region of the silk glands showed hypertrophied nuclei with polyhedra inside (Fig.50).

Wing bud:

Polyhedral bodies were visible in many cells of the wing bud, 96 hr after infection and by 120 hr most of the cells were heavily infected showing the presence of fully developed polyhedra (Fig.51).

Foregut:

Many cells of the foregut epithelium (Fig.52) at the region of cardiac valve showed polyhedra in their hypertrophied nuclei 96 hr post inoculation.

BIOCHEMICAL CHANGES IN THE LARVAE OF S.litura AFTER INFECTION BY THE NUCLEAR POLYHEDROSIS VIRUS

Studies were made on some of the biochemical changes in the larvae after infection with the nuclear polyhedrosis virus. The quantitative estimations were done at 24, 48, 72, 96 and 120 hr after inoculation of the larvae with 10^5 polyhedra per larva as described under "Materials and Methods". The results are presented below.

I. Nitrogen compounds

i) Total nitrogen

The data on the nitrogen content of healthy and NPV infected larvae at different intervals are presented in Table 9 and illustrated in Fig.53. It will be seen that the average nitrogen content in diseased larvae (12.79 per cent) was significantly higher than that of healthy larvae (11.77 per cent). In the healthy larvae, the total nitrogen content decreased with age. The maximum level of 13.07 per cent was observed at 24 hr, which decreased to a minimum of 9.89 per cent at 120 hr. But in diseased larvae, it is interesting to note that, the percentage

TABLE 9

Total nitrogen content in healthy and NPV infected larvae of S.litura at different periods after inoculation

Post-inoculation period in hour	Total nitrogen (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	13.07	12.60	- 3.59
48	12.60	13.81	+ 9.60
72	12.13	12.79	+ 5.44
96	11.15	12.60	+13.00
120	9.89	12.13	+22.95
Average	11.77	12.79	+ 8.66

C.D. (P= 0.05): Healthy Vs Diseased = 0.51

" " : Period = 0.80

" " : Interaction = 1.13

of nitrogen remained more or less at the same level during the whole period of the observation, except at 48 hr when it recorded a significant increase over that at 24 hr. No significant differences existed between the nitrogen levels at other intervals.

The data further show that the diseased larvae had a higher level of nitrogen than the healthy ones at

all intervals except at 24 hr when it showed slight decrease. However, the differences were statistically significant only at 48, 96 and 120 hr after inoculation. The most pronounced increase in nitrogen content was observed at 96 and 120 hr after treatment.

ii) Uric acid:

The data presented in Table 10 and illustrated in Fig.54 indicate that in general the uric acid content

TABLE 10

The levels of uric acid in NPV infected as compared to healthy larvae of S.litura at different intervals after inoculation

Post-inoculation period in hour	Uric acid (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	2.46	2.52	+ 2.43
48	2.25	2.40	+ 6.66
72	2.72	2.75	+ 1.10
96	1.67	2.61	+56.28
120	1.35	2.51	+85.92
Average	2.09	2.56	+22.48

C.D. (P = 0.05): Healthy Vs Diseased = 0.18
" " : Periods = 0.28
" " : Interaction = 0.39

was higher in diseased larvae (2.56 per cent) than in healthy larvae (2.09 per cent). The uric acid decreased in healthy larvae during the later stages. But no significant differences were noticeable between the uric acid contents at different periods in diseased larvae indicating that it maintained a more or less constant level during the period of observation. It is also seen that up to 72 hr both healthy and diseased larvae contained more or less similar levels of uric acid. But in diseased larvae the uric acid increased considerably during the later stages recording an increase of 56.28 per cent at 96 hr and 85.92 per cent at 120 hr post-treatment over the healthy larvae.

iii) Protein:

The results summarized in Table 11 and illustrated in Fig.55 reveal that significant differences existed between healthy and diseased larvae, the latter in general containing a higher level (60.10 mg/g) of protein than the former (50.95 mg/g). In healthy larvae the maximum content of 64.25 mg/g was observed at 120 hr, while the diseased larvae had the highest level (67.59 mg/g) at 96 hr of treatment. When the protein content of healthy and diseased larvae at the

TABLE 11

Protein content of healthy and NPV infected larvae of S.litura at different intervals after inoculation

Post-inoculation period in hour	Protein (mg/g wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	57.04	59.95	+ 5.17
48	54.22	52.59	- 3.00
72	50.99	56.95	+11.68
96	61.56	67.59	+ 9.79
120	64.25	63.38	- 1.35
Average	50.95	60.10	+17.95

C.D. (P = 0.05): Healthy Vs Diseased = 1.47

" " : Periods = 2.33

" " : Interaction = 3.29

different stages are compared, it is seen that the latter had a significantly higher level at 72 and 96 hr after treatment.

II. Total fat:

The observations recorded (Table 12 and Fig.56) reveal that the average fat content was significantly

TABLE 12

Total fat content of healthy and NPV infected larvae of S.litura at different intervals after inoculation

Post-inoculation period in hour	Total fat (mg/g wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	33.63	33.22	- 1.21
48	28.89	26.84	- 7.09
72	30.16	35.81	+18.73
96	36.26	24.31	-32.95
120	43.56	22.46	-48.46
Average	34.49	28.57	-17.16

C.D. (P = 0.05): Healthy Vs Diseased = 4.66

" " : Interaction = 10.43

higher in healthy larvae (34.49 mg/g) than in the diseased ones (28.57 mg/g). The fat content in healthy larvae showed a slight decrease at 48 hr after treatment and then started rising at subsequent intervals reaching a maximum of 43.56 mg/g at 120 hr. The diseased larvae also revealed a similar trend until 72 hr post-treatment. But thereafter it declined sharply recording

the minimum (22.46 mg/g) at 120 hr. A comparative examination of the daily fat content in both groups reveals that, at 96 and 120 hr after treatment the diseased larvae had significantly lower levels, the percentage of decrease from healthy ones being 32.95 and 48.46 respectively.

III. Carbohydrates

Healthy and NPV infected larvae were analysed for the total carbohydrates and glycogen.

i) Total carbohydrates:

The results summarized in Table 13 and illustrated in Fig.57 show that the diseased larvae had a significantly lower level of total carbohydrates, the average content being 4.17 mg/g in diseased and 5.14 mg/g in healthy. Both healthy and diseased larvae followed a similar pattern in the daily changes of total carbohydrate levels until 96 hr. During this period the carbohydrate gradually increased indicating the accumulation of the same with age. But at 120 hr post-treatment the diseased larvae recorded a sharp

TABLE 13

Effect of NPV infection on the total carbohydrate content of the larvae of S.litura as compared to normal larvae

Post-inoculation period in hour	Total carbohydrate (mg/g wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	3.57	3.42	- 4.20
48	3.55	3.68	+ 3.66
72	6.25	5.24	-16.16
96	5.27	5.05	- 4.17
120	7.06	3.43	-51.41
Average	5.14	4.17	-18.87

C.D. (P = 0.05): Healthy Vs Diseased = 0.73

" " : Periods = 1.14

" " : Interaction = 1.62

decline while there was very conspicuous increase in the comparable healthy larvae. At 120 hr, the carbohydrate content of healthy and diseased larvae were 7.06 and 3.43 mg/g respectively.

ii) Glycogen:

In general the glycogen content (Table 14; Fig.58) of diseased larvae was lower than that of healthy larvae, the net decrease being 23.68 per cent.

TABLE 14

Glycogen content of the larvae of S.litura during the course of nuclear polyhedrosis as compared to healthy larvae

Post-inoculation period in hour	Glycogen (mg/g wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	2.28	2.02	-11.40
48	0.83	0.88	+ 6.02
72	3.11	2.74	-11.89
96	1.82	1.65	- 9.34
120	3.40	1.41	-58.52
Average	2.28	1.74	-23.68

C.D. (P = 0.05): Healthy Vs Diseased = 0.48

" " : Periods = 0.75

The data show that in healthy larvae the glycogen content increased from 2.28 mg/g at 24 hr to 3.40 mg/g at 120 hr

interrupted by temporary declines at 48 and 96 hr. The diseased larvae exhibited a parallel trend until 96 hr after infection. But at 120 hr post-treatment a notable decline in glycogen content was observed in diseased larvae. Thus when the healthy larvae contained the highest level of 3.40 mg/g at 120 hr post-treatment, the diseased larvae had only 1.41 mg/g which was the lowest level ever recorded.

IV. Nucleic acids

The quantitative changes occurring in DNA and RNA in the larvae of S.litura during the course of nuclear polyhedrosis and in comparable healthy were estimated at 24 hr intervals.

i) DNA:

The daily changes in the levels of DNA in healthy and diseased larvae are presented in Table 15 and illustrated in Fig.59. It shows that the diseased larvae had significantly higher amount of DNA than healthy ones. Healthy larvae had the maximum content of DNA at 24 hr post-treatment which decreased gradually during the subsequent intervals recording the lowest at 120 hr. In infected larvae also the DNA decreased

TABLE 15

Changes in DNA content in healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	DNA (µg/mg wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	1.13	1.02	- 9.73
48	0.74	0.84	+ 13.52
72	0.78	1.62	+107.69
96	0.55	1.44	+161.81
120	0.45	1.31	+191.11
Average	0.73	1.25	+ 71.23

C.D. (P = 0.05): Healthy Vs Diseased = 0.08 ~~0.80~~

" " : Periods = 0.12

" " : Interaction = 0.18

at 48 hr. But at 72 hr it reached an altime high followed by a significant reduction at 96 hr. At 120 hr post-treatment also it declined slightly but not significantly. However, finally it had 1.31 µg DNA/mg as against 1.02 µg DNA/mg at 24 hr. The DNA level was higher in infected larvae than healthy ones at all intervals except at 24 hr post-treatment. This

increase was highly pronounced at 72, 96 and 120 hr after treatment. At 24 hr the diseased larvae recorded a slightly lower level of DNA compared to the healthy counterparts, though the difference was not significant.

ii) RNA:

The data presented in Table 16 and illustrated in Fig.60 reveal that in both healthy and diseased larvae

TABLE 16

Changes in RNA content of healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	RNA (µg/mg wet weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	6.16	4.80	-22.07
48	4.04	3.79	- 6.18
72	3.18	5.29	+66.35
96	3.79	3.38	-12.13
120	2.60	3.39	+30.38
Average	3.96	4.12	+ 4.04

C.D. (P = 0.05): Periods = 0.51

" " : Interaction = 0.72

the relative amount of RNA decreased as the larvae advanced in age. However, in diseased larvae the RNA showed a sharp increase at 72 hr after treatment and then again declined. A comparison of the RNA levels at different intervals in healthy and diseased larvae show that the latter recorded a significantly lower amount at 24 hr and significantly higher levels at 72 and 120 hr after treatment, the maximum increase (66.35 per cent) being observed at 72 hr. The difference noted at 48 and 96 hr post-treatment were not significant.

V. Minerals

The quantitative changes of calcium, magnesium, sodium, potassium and phosphorus in healthy and diseased larvae were studied.

i) Calcium:

The analyses of the calcium content (Table 17; Fig.61) showed that the diseased larvae contained a significantly low level (0.34 per cent) of calcium compared to the healthy larvae (0.40 per cent).

In healthy larvae, the calcium level increased at 48 hr then decreased at 72 hr and again increased at

TABLE 17

Changes in calcium content of healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Calcium (% of dry weight)		% increase (+) or decreased(-) over healthy
	Healthy	Diseased	
24	0.41	0.33	-19.51
48	0.44	0.40	- 9.09
72	0.31	0.35	+12.90
96	0.42	0.31	-21.42
120	0.42	0.29	-30.95
Average	0.40	0.34	-15.00

C.D. (P = 0.05): Healthy Vs Diseased = 0.03

" " : Periods = 0.04

" " : Interaction = 0.06

96 hr and maintained the same level at 120 hr after treatment. Similarly, the diseased larvae also showed an increase and decrease at 48 and 72 hr respectively. But at 96 and 120 hr the calcium level decreased markedly.

Compared to healthy larvae, the calcium content was significantly lower in diseased larvae at 24, 96 and

120 hr after treatment. The decrease was highly pronounced at 96 and 120 hr.

ii) Magnesium

The data recorded on the magnesium content are presented in Table 18 and illustrated in Fig.62.

TABLE 18

Magnesium levels in healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Magnesium (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	0.31	0.36	+16.12
48	0.35	0.34	- 2.85
72	0.39	0.35	-10.25
96	0.35	0.40	+14.28
120	0.38	0.38	-
Average	0.36	0.37	+ 2.77

It will be seen that the magnesium content did not change significantly with age or due to induction of disease.

iii) Sodium:

Statistical analysis of the data on sodium content (Table 19) in healthy and diseased larvae showed

TABLE 19

Changes in sodium content of NPV infected S.litura larvae as compared to normal larvae

Post-inoculation period in hour	Sodium (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	0.37	0.37	-
48	0.42	0.44	+ 4.75
72	0.45	0.40	-11.11
96	0.45	0.46	+ 2.22
120	0.55	0.42	-23.65
Average	0.45	0.42	- 6.66

that there was no significant difference between the two groups (Fig.63).

iv) Potassium:

The data presented in Table 20 and illustrated in Fig.64 indicate that on an average the diseased larvae

TABLE 20

Potassium content of healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Potassium (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	5.00	4.90	- 2.00
48	5.10	4.00	-21.56
72	4.77	4.27	-10.48
96	5.23	3.43	-34.41
120	3.90	2.80	-28.20
Average	4.81	3.88	-19.33

C.D. (P = 0.05): Healthy Vs Diseased = 0.26

" " : Periods = 0.41

" " : Interaction = 0.58

contained a lower level of potassium, than comparable healthy ones. The potassium content was lower at all intervals in infected larvae than healthy ones. However, the reduction was statistically significant only at 48, 96 and 120 hr after treatment. The most conspicuous reduction occurred at 96 and 120 hr, the percentage decrease from healthy being 34.41 and 28.20 respectively.

v) Phosphorus:

The data on the levels of total phosphorus in the healthy and NPV infected larvae of S.litura are presented in Table 21 and illustrated in Fig.65. It is

TABLE 21

Levels of total phosphorus in NPV infected S.litura larvae as compared to healthy larvae

Post-inoculation period in hour	Total phosphorus (% of dry weight)		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	1.17	1.07	- 8.54
48	1.20	1.18	- 1.66
72	1.27	1.15	- 9.44
96	1.18	1.17	- 0.84
120	1.12	1.23	+ 9.82
Average	1.19	1.16	- 2.52

C.D. (P = 0.05): Interaction = 0.10

seen that in healthy larvae the phosphorus content did not vary significantly until 96 hr. At 120 hr it recorded the minimum level of 1.12 per cent, which was significantly lower than the level at 72 hr. The

diseased larvae showed significant rise in total phosphorus at 48 hr and then remained in more or less the same level during the subsequent periods. When compared to the healthy larvae, the phosphorus content was lower in diseased larvae until 96 hr. However, the decrease was significant only at 72 hr. But at 120 hr, the phosphorus content in diseased larvae indicated a significant increase over healthy larvae.

TOTAL HAEMOCYTE COUNTS

The average number of circulating haemocytes in healthy and NPV infected larvae of S.litura are shown in Table 22 and illustrated in Fig.66. In general the average number of circulating haemocytes in healthy larvae increased with age with intermittant declines at alternate intervals. It increased from a minimum of 17800 haemocytes/mm^3 at 24 hr to a maximum of 33710 haemocytes at 96 hr. The diseased larvae recorded the maximum number at 24 hr and thereafter the THC decreased at subsequent intervals reaching the lowest number at 120 hr after treatment.

A comparison of the haemocyte counts of healthy and diseased larvae at the different intervals show that at 24 hr the diseased larvae had a significantly higher

TABLE 22

Average number of circulating haemocytes in healthy and NPV infected larvae of S.litura

Post-inoculation period in hour	Average number of circulating haemocytes/ mm^3 ± S.E.		% increase (+) or decrease (-) over healthy
	Healthy	Diseased	
24	17800 ± 529.34	25230 ± 1835.77	+ 41.74
48	20280 ± 350.90	18800 ± 484.19	- 56.60
72	18890 ± 617.76	15420 ± 653.73	- 18.37
96	33710 ± 1371.05	11210 ± 613.05	- 66.74
120	22180 ± 1668.16	9650 ± 613.88	- 56.49

number of haemocytes than the healthy ones. At all subsequent intervals the diseased larvae recorded significantly lower number of haemocytes than the healthy individuals.

DISCUSSION

DISCUSSION

The main objectives of the present studies have been (1) to find out the occurrence, if any, of nuclear polyhedrosis infection in the major lepidopterous crop pests of Coimbatore, Tamil Nadu and (2) to gather as much basic data as possible on the diseases detected. The main findings are discussed below:

Out of the three nuclear polyhedroses observed, that of Amsacta albistriga is recorded for the first time. The symptoms of the nuclear polyhedrosis of Amsacta albistriga generally resemble those described for other nuclear polyhedroses of lepidopterous larvae, as reviewed by Aizawa (1963) and Smith (1967). The nature of infection and the characteristics of the inclusion bodies and virus indicate that the pathogen belongs to the group Borrelinavirus causing nuclear polyhedrosis in insects. The red hairy caterpillar is a destructive pest of groundnut (Arachis hypogaea) and many other crops. Outbreaks of this pest occur annually in an epidemic form in different parts of Tamil Nadu. The caterpillars migrate in large groups from field to field devastating the entire crop. These characteristics of the larvae are quite favourable for the natural or

artificial spread of the disease. Hence field trials on the effectiveness of the virus would be worthwhile.

The symptoms of the nuclear polyhedrosis of Heliothis armigera observed in the present studies agree with those reported by Stahler (1939) and Coaker (1958). In the present studies it was observed that the diameter of the polyhedra ranged from 0.5 to 1.4 μ with an average of 0.89 μ. These measurements are in close agreement with the inclusion measurements of H.armigera by Bergold and Ripper (1957) and those of H.zea by Gregory et al. (1969). But the nuclear polyhedra of H.peltigera are comparatively larger in size (Harpaz and Zlotkin, 1965). No surface patterns were observed on the polyhedra of H.armigera in the present studies as also the case with H.zea (Gregory et al., 1969). The arrangement of the virus rods singly as observed in H.armigera is in agreement with the findings of Bergold and Ripper (1957) though these authors observed a few bundles with two rods. In Heliothis zea also the virus rods occurred singly (Gregory et al., 1969). Thus, the polyhedra and virus rods of H.armigera isolated in the present studies are similar morphologically to those of H.armigera described by Bergold and Ripper (1957) and H.zea (Gregory et al., 1969).

It was observed in the present studies that some infected larvae of H.armigera showed atypical symptoms. No previous report of such a phenomenon has been recorded in H.armigera. A more or less similar observation has, however, been reported by Harpaz and Zlotkin (1965) in H.peltigera. These latter authors observed that caterpillars of the third instar died after virus inoculation without showing any characteristic external symptoms and they concluded that this might be due to the younger larvae succumbing to an earlier stage of infection. In the present instance however, larvae infected in advanced instars also showed cases of atypical death. Little can be said now with regard to the significance of this phenomenon. Comparative studies on the histopathology of both the diseases must be made before any conclusion is made.

THE NUCLEAR POLYHEDROSIS OF THE TOBACCO CATERPILLAR *Spodoptera litura* F.

This disease was first recorded in India from New Delhi by Ramakrishnan and Tiwari (1969). But no detailed studies were reported. The present study was taken up to gather information on the host-pathogen relationship covering the symptomatology, histopathology,

nature of the pathogen, the effect of the disease on the larval size, weight, moulting and the food consumption and the biochemical changes in the larvae after infection.

In comparing the signs and symptoms, the nuclear polyhedrosis of S.litura resembles other nuclear polyhedroses of lepidopterous larvae as reviewed by Aizawa (1963) and Smith (1967). The infected larvae appeared slightly bloated in 3 days. Similar observations were made by Tanada (1954) in Pieris rapae and Steinhaus (1960) in Prodenia praefica. This might be due to the rapid hypertrophy and swelling of the epidermis noted at this stage. The pinkish colour change observed in the infected larvae may be as suspected by Steinhaus (1960) due to the accumulation of pink granules in the clear areas of the integument. It was observed in the present studies that some of the larvae which ingested polyhedra in the later instars entered the soil for pupation, but came up to surface later on and died of nuclear polyhedrosis. A similar observation was made by Vail and Hall (1969 a) in the case of Trichoplusia ni. Pre-pupal and pupal mortality as observed in S.litura have been reported by Hafez (1958) in P.litura and Tanada (1954) in P.rapae. Stairs (1965)

also observed pupal and adult mortality in G.mellonella when the larvae were fed with virus in the later stages. However, Vail and Hall (1969 a) did not find any pupal or adult mortality of T.ni when the late instar larvae were fed or injected with the virus. Their data indicated that diseased larvae seldom pupated or gave rise to adults. The emergence of malformed adults from larvae which survived virus infection and pupated were also noted by Hafez (1958) in P.litura.

The susceptibility of the larvae of S.litura to NPV infection decreased with age. Similar observations were made by Tanada (1956, 1960) in Pseudaletia unipuncta and Spodoptera mauritia and Morris (1962 a) in Lambdina fiscellaria somniaria. Further, it was noted in the present studies that lower concentrations of virus inoculum resulted in a longer incubation period. Aizawa (1953) reported that the length of incubation period depended upon both the size of the inoculum and temperature. When the temperature was constant, the lower the virus concentration, the longer was the incubation period.

The diameter of the polyhedra of S.litura was observed to vary from 1.2 to 2.8 μ with an average of 1.86 μ. The virus rods occurred singly and in bundles.

These morphological characteristics of the pathogen are similar to those reported by Bergold and Flaschentrager (1957) for Borrelina litura. The present measurements of polyhedra of S.litura are not in agreement with the inclusion measurements of P.litura which ranged from 2.5 to 4 μ (Ramakrishnan and Tiwari, 1969). The inclusion measurements of two closely related species viz., S.mauritia (Tanada, 1960) and S.exigua (Hunter and Hall, 1968) are 1.61 μ (1.07 to 3.22 μ) and 2.05 μ (1 to 6 μ) respectively.

Effect of the disease on larval size, weight and moulting:

That growth is retarded in NPV infected larvae has been reported in certain cases (Adams et al., 1968). But no precise information is available on the effect of virus infection on the larval size, weight and moulting. The present studies show that there did not exist much difference in the mean wet weight of healthy and diseased larvae until 72 hr after ingestion of polyhedra. But at 96 and 120 hr the mean weights were distinctly lower in diseased larvae compared to the healthy ones. The results thus indicate that in the diseased larvae there was a retardation of growth after the initial phase of the disease. This should naturally

be expected since the larvae fed very little on the fourth day and completely stopped feeding on the fifth day. Further the nuclear polyhedrosis is a debilitating type of disease as evidenced by the depletion of fat and carbohydrates (Table 12, 13) observed in the present study. The mean dry weight also showed a parallel trend with wet weight. The observation that the mean lengths of the diseased larvae were markedly lower than that of healthy larvae at 96 and 120 hr show further the retarding effect, the virus infection had on the growth of larvae. This finding, plus the observations on wet and dry weights would clearly show that the virus infection had a tremendous retarding effect on growth rate of the host larvae and this became more apparent after the initial signs of infection were observed externally.

Results presented also show that the healthy larvae had undergone two moultings during the period of observation while the diseased had moulted only once and the subsequent moulting was inhibited. No previous studies on inhibition of moulting by NPV infection are available. In the rickettsiosis of Melolontha melolontha, Kreig (1958) and Niklas (1960) found that except for larvae infected in the first

instar, which as a rule died in the second instar, all larvae died in the instar in which they had been infected. An interference in moulting would signify a severe interference with the growth and development of the organism. The pertinent question then is "how does the virus infection cause an inhibition of moulting?" The fact that both healthy and diseased larvae had undergone normally the first moulting which should occur within 48 hr of the start of the experiment, shows that until 48 hr the virus infection did not affect the moulting process. But the subsequent moulting, which should occur within 96 hr after the start of the experiment was completely prevented in the diseased larvae. This might indicate that the interfering factors were at work during the period between 48 to 96 hr after infection.

It is now known that moulting and metamorphosis of insects are under hormonal control. The basic pattern of hormonal control in moulting as given by Gilbert and Schneiderman (1961 a) involves, the activation of prothoracic glands by a brain hormone secreted by the neurosecretory cells of the brain. The activated prothoracic glands respond to this stimulus by elaborating and releasing a hormone, the prothoracic

gland hormone, which reacts with various cells of the insect and starts them growing. In the case of epidermis, the prothoracic gland hormone activates its synthetic machinery to deposit a new cuticle and thus initiates the moulting process.

It was observed in the present studies, that the brain, ventral nerve ganglia and nerve cords were infected. Morris (1970) had observed that there was an apparent reduction in the amount of neurosecretion in the brain after NPV infection in Lambdina fiscellaria somniaria and Orgyia pseudotsugata. Thus, it is possible that in the present instance also, the infection of the brain interfered with the neurosecretion. Further, the infection observed in the ventral nerve ganglia and nerve cord was likely to affect the hormonal balance of the insect because these tissues regulate indirectly the activity of corpora allata through the neurosecretory system (Scharrer and Scharrer, 1963). Moreover, the hypodermis had been completely inactivated by the virus infection, thus rendering it non-responsive to activation by the prothoracic glands. Further, according to Locke and Collins (1967) the hypodermis is continuously taking up blood proteins and it does so most intensively when

its growth and cuticle deposition are highest. Nuclear polyhedrosis infection has been reported to cause a decrease of haemolymph protein concentration in several cases (Martignoni and Milstead, 1964; van der Geest and Craig, 1967; van der Geest and Wassink, 1969; Shapiro and Ignoffo, 1971; Young and Lovell, 1971). This may be happening in the NPV infected Spodoptera larvae also. Thus, it is also possible that the moulting might have been inhibited, at least in part, due to lack of sufficient blood proteins to feed the hypodermis for the formation of new cuticle. Further the infection of many haemocytes and the reduction in the total haemocytes observed in the present studies also might have interfered with the moulting process as according to Wigglesworth (1955) the haemocytes may play some role in the interaction between the brain and prothoracic gland. Thus, it may be concluded that the above mentioned factors individually or collectively brought about the inhibition of moulting in the NPV infected larvae of S.litura.

Food consumption of the larva:

The observations on the leaf consumption of healthy and diseased larvae revealed two interesting

phenomena. The virus infection resulted in an enhanced feeding on the second day. On the other hand, it caused a retardation of feeding on fourth day and complete cessation of feeding on the fifth day. Though loss of appetite and cessation of feeding have been reported as a common symptom associated with nuclear polyhedrosis infections (Aizawa, 1963; Smith, 1967), no quantitative data are available on this aspect. Histopathological studies had revealed that the active virus multiplication started on the second day after ingestion of polyhedra. It might be that to counteract the heavy demands forced upon it by the active viral synthesis, the larvae were attempting to consume more food as a compensatory function. But the virus had overcome the animal systems in the course of infection causing a general physiological debility which indirectly would have been responsible for the retardation and cessation of feeding in the later stages.

HISTOPATHOLOGY

The fat body, hypodermis, tracheal matrix and blood cells are the major tissues infected by nuclear polyhedrosis viruses (Aizawa, 1963; Smith, 1967). The present studies showed that the above mentioned tissues were the major sites of infection in the nuclear polyhedrosis of S.litura also.

In addition to the tissues mentioned already, light to moderate infection was also observed in the brain, ganglia, neurilemma of nerve cord, muscle cells, connective tissue surrounding the midgut, silk glands, wing bud and foregut of S.litura. Infection of these tissues have been reported previously in a few other Lepidoptera. Tanada (1959 b) observed infected muscle cells in Pseudaletia unipuncta. Benz (1963 b) listed nerve sheath, ganglion cells and muscle cells among the tissues infected by the nuclear polyhedrosis of. Malacosoma alpicola. Stairs (1965) found infection of wing discs of B.mori, Choristoneura fumiferana and G.mellonella. In NPV infected Zebra caterpillar, Ceramica picta, Adams et al. (1968) observed polyhedra in the connective tissues surrounding the ganglia, nerve cord and brain and they suspected that viral replication was occurring in the nuclei of the tracheal matrix associated with these organs and tissues. Hamm (1968) reported infection of the epithelial layer of foregut, imaginal wing discs, brain, silk glands and ganglionic cells in Spodoptera frugiperda. Mathad et al. (1968) also found infection of neurilemma of nerves, ganglia, brain, sarcolemma, silk glands, connective tissue surrounding the midgut and wing buds in Trichoplusia ni.

Vail and Hall (1969 b) observed infection of muscle and ganglia in T.ni infected with a nuclear polyhedrosis virus of the SEV type.

The course of infection of various tissues of S.litura follows very closely that reported by Hamm (1968) in S.frugiperda. In the latter insect all the susceptible tissues showed polyhedra in their nuclei by 72 hr after inoculation. The present studies also showed that a similar infection of tissues except, the wing buds and foregut which showed polyhedra only 96 hr following inoculation. However, the fat body was in an early stage of infection at 72 hr and only few nuclei had polyhedra inside, contrary to that reported in S.frugiperda.

A definite sequence of infection of the various tissues of S.litura could not be established. However, Benz (1963 b) could observe the following sequence in time, of development of polyhedra in the larvae of Malacosoma alpicola: (1) fat body, (2) hypodermis, (3) tracheal matrix, (4) muscular sheath, (5) nerve sheath, (6) muscles, (7) ganglia and (8) pericardial cells.

The condensation of chromatin observed in the nuclei of fat body and hypodermis of S.litura, apparently is what Xeros (1956) described as Virogenic stroma.

The appearance of large numbers of polyhedra in the hypodermal cells throughout the body coincides with the time when the first sign of infection were discernible externally. At this time the cuticle took on an oily appearance and assumed a whitish or pinkish tinge. This may, perhaps, be due to the accumulation of large number of polyhedra in the underlying hypodermis. Further, it was also observed that the infected larvae failed to moult 3 days after inoculation. The significance of this phenomenon in relation to the hypodermal infection have been discussed earlier.

The nature of infection in the hypodermis of S.litura differ from the previously reported cases. Though hypodermis has been found to be a major site of infection in lepidopterous larvae, the highly conspicuous thickening and stratified appearance as observed in the present studies is not a common feature. However, Watanabe (1968) reported an abnormal proliferation of cells in the epidermis of the fall webworm, Hypandtria cunea induced by infection of a

nuclear-polyhedrosis virus and the newly formed cells due to proliferation were also infected immediately. Though it was not possible in the present studies to verify the phenomenon of a proliferation of cells, the stratified appearance gave an indication to that effect.

A striking feature of the infection of the fat body of S.litura was its slow rate and lack of uniformity. Full infection of the fat body was observed only by 120 hr after inoculation by which time the hypodermis and tracheal matrix had started disintegration. But it is interesting to note here that chromatin condensation of some fat body nuclei was visible 48 hr after inoculation along with such developments in the nuclei of hypodermal cells. Further, even adjacent cells of fat body showed quite different stages of infection. Such lack of uniformity of infection was also reported by Harpaz and Zlotkin (1965) in the fat body of H.peltigera. In their study on the histopathology of Spodoptera exigua nuclear polyhedrosis Hunter and Hall (1968) also found advanced symptoms of infection in the hypodermis and trachea while the fat cells had not yet begun polyhedron formation. These observations give rise to the questions; whether

different cells or regions of fat body vary in their susceptibility to virus infection or is it a chance occurrence only? or whether fat body is less susceptible than hypodermis and tracheal matrix? It may perhaps be that, as suggested by Hunter and Hall (1968), the infective agent either invades susceptible tissues at random or that cells of the trachea and hypodermis have inherent properties which enable the infection process to progress at a faster rate. Benz (1963 b) found that the fat body situated next to the midgut were the first to show symptoms and he suggested that after passage through the gut wall the infective virus units were quickly absorbed by the neighbouring cells.

The reduction of fat vacuoles in the early stages and their disappearance in many areas in the final stages indicated a depletion of stored fat. Biochemical studies on total fat content (Table 12) also showed that the infected larvae had very little fat in the final stages compared to healthy larvae of the same age.

In the present studies, no infection of hindgut, gonads, Malpighian tubes or pericardial cells were observed, though infection of these tissues have

been reported in closely related species, S.frugiperda by Hamm (1968) and S.mauritia by Tanada (1960). This may be due to the difference either in the nature of the infecting viruses, nature of the host species or both. It is also important to consider the effects of the age and stage of the host at the time of infection and the amount of inoculum.

BIOCHEMICAL CHANGES IN THE NPV INFECTED LARVAE

Nitrogen compounds:

The observation that the healthy larvae of S.litura showed a decrease in total nitrogen content with age is in agreement with those of Rudolfs (1926) in Malacosoma americana and Rainey (1938) in Lucilia sericata.

The NPV infected larvae had a more or less constant level of total nitrogen during the period of infection. Thus, comparing the healthy and diseased larvae of the same age there is higher nitrogen content in the latter than in the former, this difference becoming more pronouced when the disease advances. Similar increase in total nitrogen had been observed in B.mori larvae infected by nuclear polyhedrosis virus

(Tarasevich, 1952). The increased nitrogen content of the diseased larvae may be due to (1) preservation of large quantities of nitrogen in the form of polyhedral protein since polyhedra consists of 95 per cent protein (Bergold, 1963) and (2) the retention of comparatively higher concentrations of uric acid which is a nitrogen rich compound.

That the uric acid content in NPV infected larvae of S.litura was higher than that in healthy larvae is consistent with that reported in the larvae of Neodiprion swainei by Smirnoff and Valero (1969). It was also higher in the adipose tissue of densonucleosis infected larvae of Galleria mellonella (Smirnoff and Loiselle, 1969).

Uric acid may be formed as the end product of protein and purine catabolism (Chefurka, 1965a) and/or may arise by synthesis from other nitrogenous wastes (Wigglesworth, 1965; Patton, 1963). It may thus be presumed that nuclear polyhedrosis infection which is accompanied by an enhanced protein and nucleic acid metabolism result in an increased production of uric acid in the host cells. Smirnoff and Loiselle (1969) also suggested that, the increased uric acid observed

by them in the adipose tissues of densonucleosis virus infected G.mellonella larvae reflected a physiological alteration directly related to purine metabolism.

It may be appropriate to recall here some of the physiological functions attributed to uric acid other than excretory. Ludwig (1954) suggested that it may act as a source of nitrogen, particularly during the pupal period. Studies by Mitlin and Mauldin (1966) indicated that uric acid in the boll weevil (Anthonomus grandis Boheman) is metabolically active and serves as a source of nitrogen. Ross (1959) suggested that uric acid may be involved in the synthesis of nucleoproteins. The larvae infected by the nuclear ppolyhedrosis virus are under heavy demands for nitrogen required for the synthesis of viral nucleoprotein and polyhedral protein. Thus, it is quite reasonable to presume that the increased uric acid content observed in diseased larvae has some such physiological role also which needs experimental evidence.

The finding that the NPV infected larvae of S.litura contained a higher level of total protein corresponds to observations on the silkworm, B.mori infected by NPV (Bergold and Friedrich-Freksa, 1947). These authors found that in the diseased silkworm the

protein concentration was higher (5.4 per cent) than in healthy ones (3.4 per cent). Shigematsu and Takeshita (1958) reported that the total protein in the fat body and body fluid increased in the later stages of nucleo-polyhedrosis of B.mori larvae. Later studies (Watanabe, 1967; Shigematsu and Noguchi, 1969; Watanabe and Kobayashi, 1969; and Morris, 1971) on protein synthesis in NPV infected insects also showed that the total protein content of diseased larvae was higher than that of normal larvae.

It is now almost generally agreed that virus infection of plants should be regarded as a change in the nucleoprotein metabolism of the host (Bawden, 1959). The same may be construed true in the case of virus infection in animals also since, as remarked by Smith (1967), "virus is a virus regardless of its derivation". The change in nucleoprotein metabolism should have a still wider relevance in the case of nuclear polyhedrosis infections, where the virus rods themselves are occluded in a higherly protein rich crystals.

The conspicuous increase in total protein observed in NPV infected S.litura larvae at 72 and 96 hr after treatment is evidently due to the increased protein

content resulting from active synthesis of protein leading to the formation of millions of polyhedra.

It was also observed in the present investigations that at 120 hr post-inoculation there was not much difference between the total protein contents of healthy and diseased larvae. The healthy larvae by this time were in the final instar preparing to enter the pupal stage, when the synthesis of blood proteins is maximum (Wyatt, 1961; Wigglesworth, 1965). The diseased larvae on the other hand, were in an advance stage of infection and the active synthesis of viral protein might have slowed down. Thus, when the normal larvae were actively synthesizing the protein required for their next stage of growth, the diseased larvae were in a more or less inactive and weak stage wherein all the metabolic activities had come to a standstill.

Total fat:

The pattern of fat accumulation observed in the healthy larvae of S.litura follows that reported in B.mori (Niemierko et al., 1956), Pieris rapae and Pieris napi (Strogaya, 1961) and Hyalophora cercopia (Gilbert and Schneiderman, 1961 b), wherein the fat content increased sharply in the later instars.

The fat content of diseased larvae was observed to decrease markedly in the later stages of infection viz., 96 and 120 hr after treatment when compared to the levels in corresponding periods of healthy larvae. The results also showed that in the diseased larvae the fat content decreased from 72 hr onwards and no further increase was observed. These alterations indicate that, not only the infected larvae did not accumulate fat after 72 hr of infection but also that, even the stored up fat itself was being exhausted in the later stages. Studies on the effect of NPV infection on the fat metabolism of insects are very few. Mamedniyazor et al. (1966) reported that jaundice infection of the silkworm, B.mori caused some qualitative and quantitative changes in the lipids viz., a decrease in the total lipid level and an increase in free fatty acid level, the changes being noted in the fifth and sixth days after infection while there were no differences on the third day (before the appearance of jaundice). It is reported that a considerable number of insects utilize fat as an energy source during starvation or quiescence (Fast, 1964). In the present instance, the diseased larvae could have very little or no dietary source of fat as they fed very little on the fourth day and stopped feeding on the fifth day (Table 6). Thus, starvation

may account for the fat depletion noted in the present studies. Now it is clearly known that insects can synthesize fat de novo from carbohydrates (Fast, 1964). The possibility of this type of transformation in the present case is however, doubtful because as is evident from the histopathological studies the fat body, the main site of synthesis and storage of fat, itself was infected and that the infection reached the maximum intensity in the last two days. The fat bodies weakened by the virus infection can not accomplish its main function of synthesis and storage of fat. So in the absence of any dietary source of fat and with an adipose tissue system that has been weakened and disorganized by the virus infection, the stored up fat itself was being mobilized for life purposes.

Carbohydrates:

The diseased larvae had in general a low level of carbohydrates compared to the healthy counterparts and the difference between them was highly conspicuous at 120 hr, the diseased larvae recording a decrease of 51.41 per cent over the healthy larvae. Studies on the effect of NPV infection on carbohydrates in insects are rather few. Darren Kuang et al. (1966)

observed a depletion of total sugars in NPV infected P.ricini Boisduval.

The depletion of carbohydrates may be due to virus-induced metabolic alteration, to other factors or to both. In the present experiments, the infected larvae fed very little on the fourth day and stopped feeding on the fifth day resulting in a state of starvation in the final stages. Thus, starvation is a possible explanation for the observed loss of carbohydrates as observed by Mellanby (1932) in Tenebrio molilor. Further glycogen, one of the major fractions of carbohydrates in insects also showed a drastic depletion in the larvae in the final stages of infection (Table 14). This depletion will definitely reflect in the total carbohydrates level. Thus, decreased food intake, the virus induced metabolic alterations and the depletion of glycogen appear to account for the low level of carbohydrates in the NPV infected larvae of S.litura.

The accumulation of glycogen during growth with temporary declines at moulting periods as observed in the healthy larvae of S.litura is similar to the pattern reported in silkworms, B.mori (Zaluska, 1959), Antheraea pernyi (Smolin and Gudalina, 1957; Pravdina

and Smolin, 1958) and Hyalophora cercopia (Bade and Wyatt, 1962). According to Zaluska (1959) the glycogen falls sharply during moulting in B.mori presumably because of cessation of feeding and the demands for chitin synthesis.

The diseased larvae of S.litura recorded a low level of glycogen at 120 hr after treatment and the reduction was about 58.52 per cent over the healthy counterparts. It must be noted here that the healthy larvae had just completed a moulting by 96 hr while the diseased ones did not undergo the corresponding moulting. Thus, even without a moult, the glycogen showed a decrease at 96 hr. Morris (1962 b) from histochemical studies on NPV infected fat body of the western oak looper, Lambdina fiscellaria somniaria also reported a drastic reduction of glycogen between the fourth and fifth day following infection. Sturtevant (1924) found that bees dead from foulbrood contained almost no glycogen. Comparable alterations of glycogen metabolism have been demonstrated for mammalian viruses and bacteria by Racker (1954), Gaylord (1958), Erman (1959), Kigoshi et al. (1959) and Bearcroft (1960).

The pertinent question, however, is whether the glycogen depletion as observed in S.litura is due

to virus-induced metabolic derangement, to some other factors or both. Rockstein (1950) in his review on glycogen metabolism in insects showed clear evidence that the glycogen content of normal caterpillars increased with age. It is also known that starvation brings about depletion of glycogen in insects (Mellanby, 1932; Steinhaus, 1949 a; Baude, 1967). In the present experiment feeding by the diseased larvae was at a low level on fourth day and practically nil on the fifth day following infection. As regards depletion of glycogen also, starvation is thus a possible explanation. Recent studies clearly indicate that insects possess enzymatic mechanisms for the synthesis of glycogen from monosaccharides (Chefurka, 1965b). Bergold (1959) mentions that "the hexokinase activity on glucose and fructose in the fat body of B.mori appears to be decreased at the onset of symptoms of polyhedrosis." Thus, it is also possible that the glycogen depletion resulted (at least in part) from an interference with enzymatic activity. Yet another possibility is the dysfunction in endocrine mechanisms. Wyatt (1967) stated that in insects, as in the vertebrates, the regulation of carbohydrate metabolism is effected in part by hormonal mechanisms. Histopathological evidences indicate that some dislocation in endocrine

system has been brought about by virus infection. It is thus possible that starvation and virus induced metabolic alterations through its interference on enzymatic and hormonal mechanisms, individually or collectively might have brought about the depletion of glycogen.

Nucleic acids:

The decrease in the relative amounts of the two nucleic acids noted in the healthy larvae of S.litura as they advanced in age is in agreement with that reported by Niemierko et al. (1956) in G.mellonella.

The present experiment indicated an increase of total DNA in the diseased larvae from 72 hr after inoculation. Similar increase of DNA in NPV infected B.mori larvae was reported by Tarasevich (1954) and Yamafuji et al. (1954). Most of the recent studies report the synthesis of nucleic acids in separate tissues of the infected insects and as such they are not directly comparable with the result of the present studies. However, these later studies show that DNA synthesis in the infected tissues increased progressively up to a point prior to the completion of viral inclusion formation (Morris, 1962 b, 1968, a, b; Benz, 1963 a; Shigematsu

and Noguchi, 1969 a, b, c). In the present investigations polyhedra were observed in the tissues from 72 hr after infection. The total DNA content was also found to increase from this time onwards. The increase in total DNA is thus clearly the result of DNA synthesized and incorporated in the virus rods.

The RNA in virus infected larvae of S.litura showed a decrease at 24 hr and an increase at 72 and 120 hr after treatment. This is not in agreement with the findings of Yamafuji et al. (1954) and Tarasevich (1954) that RNA decreased in NPV infected silkworm, B.mori. But later studies by Morris (1966) and Shigematsu and Noguchi (1969 a, b, c) showed an increase of RNA synthesis in susceptible tissues. However, the exact significance of the alterations noted in total RNA of the NPV infected S.litura larvae is not clear.

Minerals

Results presented revealed marked alterations in the levels of only calcium and potassium in the virus infected larvae. The phosphorus content was altered only at certain intervals after treatment.

The calcium content decreased in infected larvae. Such a phenomenon has not been previously

shown for virus-infected insects. Comparable alterations of calcium content have been demonstrated for plant viruses by Wharton (1957) in TMV-infected tomato, Narayanaswamy (1963) in PSMV-infected pigeon pea leaves and Jeyarajan (1965) in PVY-affected chillies. Gershenson (1958) reported that addition of cobalt sulphate (0.05 per cent) to the food of B.mori larvae decreased the frequency of spontaneous polyhedrosis, particularly if calcium chloride (1 per cent) was added.

The observation that potassium decreased in NPV infected Spodoptera larvae is in consonance with that of Akune (1951) in B.mori. Arseniev and Bromley (1951) reported that insufficient amounts of potassium in the oak leaves favoured the outbreak of polyhedrosis in Antheraea pernyi larvae.

The reason for the decline of calcium and potassium in the diseased larvae is obscure. However, in the light of the findings of Arseniev and Bromley (1951) and Gershenson (1958), the present observations on calcium and potassium in virus infected larvae, perhaps, give an indication that calcium and potassium act as factors increasing the susceptibility of larvae to virus infection. But further studies are required before any conclusion is made.

The diseased larvae, in the present studies, generally had a lower phosphorus content until 96 hr after infection, though the difference was significant at 72 hr only. Tarasevich (1952) found that polyhedrosis infected B.mori larvae were deficient in phosphorus. The increase in total phosphorus level observed in the present instance at 120 hr may be the result of the increased level of DNA and RNA recorded.

TOTAL HAEMOCYTE COUNT

The present studies showed that in the healthy larvae, the average number of circulating haemocytes increased with age interrupted by marked decreases at premoulting periods. Similar observations have been made by Patton and Flint (1959) on the THC of nymphs of the American cockroach, Periplaneta americana L. and Nittono (1960) on the THC of the silkworm, B.mori. In the present studies the healthy larvae were in the premoulting stage at 24 and 72 hr and at 120 hr, they were nearing the pre-pupal stage.

The diseased larvae were in the premoulting stage at 24 hr as their healthy counterparts. But contrary to that in healthy larvae, the diseased larvae showed a significantly higher number of haemocytes.

Such increase in THC has been reported by Beard (1945) in Japanese beetle grubs (Popillia japonica) infected with milky disease bacteria and by Sussman (1952) in the silkworm, Platysamia cercopia infected with the fungus Aspergillus flavus. Zelinskava (1968) found that at an early stage of polyhedrosis of Porthetria dispar, proleucocytes, macroleucocytes and active phagocytes appeared. It might be that the entry of virus into the haemolymph stimulated mitotic division of haemocytes as a protective function. Since many cells are capable of phagocytosing free virus rods as well as polyhedra as demonstrated by Kislev et al. (1969) in S.littoralis, it would be to the animals' advantage to increase the number of phagocytes by mitosis under such conditions. Tauber (1940) observed that the presence of two bacterial pathogens in the blood of Blattella orientalis stimulated mitotic division. However, no such increase in THC was noted in the NPV infected wax moth (G.mellonella) larvae in the early stages of the disease (Shapiro, 1967). Further, Shapiro et al. (1969) also did not observe any difference in the THC of healthy and NPV exposed bollworms (H.zea) for the first 3 days.

In the present studies it was also observed that the THC steadily decreased in the infected larvae from 48 hr onwards, recording a decrease of 67 and 56 per cent respectively from the corresponding healthy group at 96 and 120 hr after treatment. Shapiro et al. (1969) also noted a drastic reduction of THC in H.zea larvae after exposure for three days to a high dose of nuclear polyhedrosis virus, but not in healthy or in larvae exposed to a low dosage. In the larvae of G.mellonella infected with a nuclear polyhedrosis, Shapiro (1967) observed a significant decrease in THC from 10 days after treatment. However, in none of these earlier studies such a quick and marked decline in THC, as observed in S.litura, were recorded. It is to be emphasized here, that the disease in S.litura larvae developed rather quickly killing them within 6 days of infection. Further, polyhedra were visible in the nuclei of many haemocytes from 72 hr onwards and thereafter the number of disintegrating cells were on the increase. The destruction of the cells, perhaps, may account for a major percentage of decrease. Further, Jones (1962) suggested that in most insects during larval life the haemocytes increased principally by mitotic division of the circulating haemocytes. It is possible that nuclear infection of blood cells, as observed in the present experiment, interfered with the normal mitotic divisions.

SUMMARY

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Nuclear polyhedroses were observed in three species of major lepidopterous crop pests viz., (i) the red hairy caterpillar, Amsacta albistriga W., (ii) the gram pod borer, Heliothis armigera Hb. and (iii) the tobacco caterpillar, Spodoptera litura F.

Nuclear polyhedrosis of A.albistriga W.

1. The nuclear polyhedrosis of A.albistriga is recorded for the first time.

2. The infected larvae of A.albistriga exhibited loss of appetite, became sluggish and turned dirty brown in colour within 5 to 6 days after ingestion of polyhedra. Death occurred in 8 to 10 days with liquefaction of internal tissues. At death the cuticle ruptured liberating the liquefied body contents.

3. The polyhedra measured on an average 1.19 + 0.015 μ in diameter with a range from 0.7 to 1.7 μ. No surface patterns were apparent over the polyhedra. The virus rods occurred singly and in bundles of 2 to 5 rods.

4. The characteristics of the pathogen indicate that it belongs to the group Borrelinavirus causing nuclear polyhedroses in insects.

Nuclear polyhedrosis of H.armigera

1. Pathogenicity tests showed that the nuclear polyhedrosis of H.armigera was highly infectious.

2. The symptoms of H.armigera nuclear polyhedrosis were similar to those reported earlier. Death occurred in 5 to 9 days. Dead larvae showed the characteristic 'hanging' position.

3. Some of the larvae which ingested the polyhedra died without showing the typical symptoms though tissue and haemolymph smears showed the presence of polyhedra.

4. The diameter of the polyhedra ranged from 0.5 to 1.4 μ with an average of 0.89 μ. The polyhedra showed no surface patterns. The virus rods occurred singly.

5. The morphological characteristics of the pathogen closely agree with those described by Bergold

and Ripper (1957) for Borrelina armigera and to those of the polyhedra and virions of H.zea as described by Gregory et al. (1969).

Nuclear polyhedrosis of S.litura F.

1. The symptoms of the disease resembled those reported already for the nuclear polyhedroses of other lepidopterous larvae.

2. The larvae infected in the earlier instars assumed a greenish or greenish yellow paller and those infected in the later instars turned pink or orange in colour.

3. Larvae died over the surface of the leaves or in the classical 'hanging' position.

4. The susceptibility of the larvae decreased and the incubation period increased with age.

5. The average incubation period of the disease when infected with a dosage of 10^6 polyhedra per larva varied from 4 days for the second instar to 7.7 days for the sixth instar.

6. Fourth instar larvae when infected with 10^3 and 10^4 polyhedra per larva had mean incubation periods of 6.5 and 6.3 days respectively with mortality of 44 and 66 per cent. At dosages of 10^5 and 10^6 polyhedra per larva, these were 5.8 days, 5.3 days and 100 per cent respectively.

7. The polyhedra measured on average $1.86 \pm 0.025\ \mu$ in diameter with a range from 1.2 to $2.8\ \mu$. No surface pattern was apparent for the polyhedra.

8. The virus rods occurred singly and in bundles of 2 to 14 rods.

9. The characteristics of the polyhedra and virus rods resembled closely those of Borrelina litura described by Bergold and Flaschentrager (1957).

10. The infected larvae recorded low wet and dry weights on the fourth and sixth day of infection compared to healthy larvae of the same age.

11. The diseased larvae did not increase in length after 3 days of ingestion of the polyhedra and they were significantly shorter than healthy larvae on fourth and fifth days.

12. In infected larvae moulting after 3 days of infection was found to be inhibited.

13. The diseased larvae consumed a significantly larger area of leaf on the second day following ingestion of polyhedra. Feeding was at a slower rate on the fourth day and ceased completely on the fifth day.

14. Hypodermis, fat body, tracheal matrix and blood cells were the major sites of infection.

15. Light to moderate infection were also noticed in brain, silk glands, foregut, ventral nerve ganglia, neurilemma of nerve cord, muscles and imaginal wing buds and the connective tissue surrounding the midgut.

16. No signs of infection were apparent under light microscope 24 hours after inoculation.

17. At 48 hours most of the cells of hypodermis and some cells of the fat body showed hypertrophied nuclei with condensed chromatin material at the centre.

18. At 72 hour post inoculation polyhedra were distinctly visible in the nuclei of most of the cells of hypodermis and tracheal matrix and in some nuclei of

fat body, blood cells, nerve ganglia, neurilemma, brain and muscle tissues, silk glands and the connective tissue surrounding the midgut.

19. By 96 hours the infection had greatly advanced in hypodermis, tracheal matrix and blood cells and to some extent in the fat body. Further, some cells of the foregut and wing bud also showed presence of polyhedra.

20. At 120 hours all the tissues were in an advanced stage of infection and signs of disintegration were visible.

21. The infection appeared more rapid and uniform in hypodermis and tracheal matrix than in fat body.

22. Another feature of the infection was the abnormal thickening of the hypodermis.

23. The diseased larvae had a higher level of total nitrogen over comparable healthy ones at all intervals except at 24 hour. The increase was more pronounced at 48, 96 and 120 hours.

24. The uric acid content was always higher in diseased larvae, the difference being more pronounced at 96 and 120 hours of inoculation.

25. There was a significant increase in total protein content of diseased larvae over the healthy ones at 72 and 96 hours.

26. The diseased larvae had significantly lower level of total fat at 96 and 120 hours after inoculation compared to the healthy larvae.

27. The total carbohydrate and glycogen content decreased in diseased larvae and the reduction was more pronounced at 120 hours post-inoculation over comparable healthy ones.

28. DNA was significantly higher in diseased larvae at 72, 96 and 120 hours after infection.

29. RNA content was significantly low at 24 hours and high at 72 and 120 hours after treatment in diseased larvae than in the healthy ones.

30. The diseased larvae contained a lower level of Ca and K compared to the healthy larvae.

31. Sodium and magnesium content were not altered due to the infection.

32. The total P was significantly lower at 72 hours while it was conspicuously higher at 120 hours

in the diseased larvae compared to the healthy ones.

33. The infection caused marked alterations in the number of circulating haemocytes. It caused an increase over the healthy larvae at 24 hour after inoculation followed by significant reductions at the subsequent intervals.

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