GENERATION TO GENERATION TRANSMISSION OF MICROSPORIDIA WHICH INFECT INSECTS

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ABSTRACT

Generation to generation transmission via the eggs has been studied in 4 microsporidian/host systems.

In corn earworms, Heliothis zea, infected with Nosema heliothidis meronts and sporonts of the parasite invaded the ovarian tissue. Spores were seen throughout the ovarian tissue but they were degenerate. Embryonated eggs contained a few well developed spores in several tissues of the embryo and in the meconium which filled the lumen of the gut. Generation to generation transmission via the eggs was highly efficient, so that usually 100 per cent of the progeny of infected females were already infected, when they hatched from the eggs. Although the male reproductive system was invaded by N. heliothidis in the testicular cyst cells, there was no transmissin to the eggs during fertilization.

Two other microsporidia, Nosema bombycis a natural parasite of silk worms, Bombyx mori, and Pleistophora operophterae a natural parasite of winter moth, Operophtera brumata, were also investigated in H. zea. Both invaded the reproductive tissue of male and female hosts. Infection was heavy and the infected adults failed to emerge from the pupae. Meronts, sporonts, and well developed spores were seen in the ovarian tissue and oocytes. In these two systems transovarial transmission could occur but there was such a low survival rate that it could not be regarded as an effective mechanism for transmission to the next generation.

Again there was no evidence of transmission to the eggs via the male at fertilization.

The fourth parasite Nosema locustae was investigated in several orthopteran hosts: Locusta migratoria, from which it had first been described and Oxycatantops praemonatus, Oxycatantops annulipes and Catantops sylvestrii which are new host records. In L. migratoria the microsporidian invaded only the epithelial sheath of the ovariole and intertubular connective tissue of the testis and there was no spread into eggs. Larvae hatching from the eggs were entirely free of infection. The ultrastructure of the various stages of the parasite, not previously described, was studied. In the new hosts there was no invasion into the reproductive tissue.

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GENERAL INTRODUCTION

The phylum Microspora comprises a group of obligate intracellular parasites with very characteristic spores for transmission. The spore wall is composed of two easily discernible layers closely adherent to each other. The thick inner spore coat is normally of very low electron density, whereas the relatively thin outer coat is characteristically opaque to electrons. The wall encloses the nucleus and cytoplasm of the future sporoplasm and an extrusion apparatus consisting of a stack of membranes known as the polaroplast, the polar tube and posterior vacuole. It has been suggested that swelling of the polaroplast and posterior vacuole, under certain stimuli, creates an internal pressure which causes the polar tube to be everted like the finger of a glove (Lom and Vavra, 1963). However, Weidner (1972), Weidner and Byrd (1982) and Weidner, Byrd, Scarborough, Pleshinger and Sibley (1984) have proposed a model for polar tube assembly outside the spore and have suggested that the polaroplast provides the plasma membrane for the emergent sporoplasm. Once the polar tube has been discharged from the spore, the infective agent or sporoplasm travels through the tube and, if the tip of the tube has penetrated the membrane of a host cell, the sporoplasm is inoculated into the host cell to initiate an infection.

On reaching its site of development in a new host, there are repeated cycles of binary or multiple fission (merogony) followed by another division process, sporogony, which gives

rise to spores. Repeated merogonies culminating in sporogony lead to the almost total replacement of host tissues by spores. The infected tissues become opaque and whitish in colour. The effect of microsporidia on hosts can be related to malfunction of organs by overburdening numbers rather than toxicity. The high reproductive potential of microsporidia and destruction of host tissues frequently results in mortality or lowered reproductive performance of the host.

Infection usually begins when the spores are ingested and hatch in the gut but many lepidopteran species are capable of passing on the infection directly to the next generation: the parasites pass into the eggs but generally do not prevent the resulting larvae from developing. Thus, the retarded development of microsporidia in insect eggs, compared with the more rapid development in larvae and adults, makes an interesting contrast.

Several aspects of microsporidian biology have been investigated in the present work:

a) The transfer of infection from parent to offspring in several insect host/microsporidian parasite systems.

b) The pathogenicity of Nosema heliothidis in a susceptible and economically important lepidopteran host, the corn earworm Heliothis zea, both in the parent and F_1 generations, with similar studies of Nosema bombycis and Pleistophora operophterae in the same host. The natural host of N. bombycis is in the silk worm, Bombyx mori and that of P. operophterae is the winter moth, Operophtera brumata.

c) The susceptibility of the grasshoppers Oxycatantops praemonatus, Oxycatantops annulipes and Catantops sylvestrii to Nosema locustae. This microsporidian species, in which no transmission via the egg has been observed, has also been investigated in locusts, Locusta migratoria migratorioides in order to determine whether the infections pass into the reproductive tissues. N. locustae has not been investigated previously at the ultrastructural level and observations on its fine structure, obtained during examination for signs of infection in the reproductive tissues are also included.

1. REVIEW OF THE LITERATURE

1.1 The pathogenicity of microsporidia with particular reference to Lepidoptera.

1.1.1 Mechanisms of pathogenicity

Most of the pathogenic effects of microsporidia are related to a reduction in the food reserves in the fat tissue. This may be due to a direct distruction of fat cells by the parasites or to a failure by the host to replenish the reserves, when they are withdrawn for energy conversion. The midgut is the primary site of infection of Nosema pyraustae which infects Pyrausta nubilalis (=Ostrina nubilalis) (Van Denburgh and Burbutis, 1962), of Nosema fumiferanae which infects Choristoneura fumiferana (Thomson, 1958a) and Nosema heliothidis which infects Heliothis zea (Kramer, 1959b). The midgut is important in digestion and absorbtion of food and cells packed with spores function inefficiently compared to healthy ones. In infected larvae there is a reduction in the functional area of the gut, the amount of reduction depending on the degree of infection. Because of this, the amount of fat tissue is less in the infected larvae than in the uninfected ones. Pleistophora oncoperae infects the musculature and fat body of Oncopera alboguttata (Milner, 1977) and N. locustae infects the fat body of its orthopteran hosts (Canning, 1953). In these hosts the fat body reserves are greatly reduced by the parasite. As the adult stage in Lepidoptera is entirely

dependent on the fat body reserves, and in Orthoptera reserves in the fat body must be replenished by absorbtion from the gut, it follows that the longevity of adults will be affected. As females convert a large portion of their reserves into yolk in the formation of eggs, the effect is more noticeable than in males.

1.1.2 Pathogenicity

Numerous studies have shown significant larval mortality and reduction of adult longevity and fecundity in various insects infected with microsporidia. Some of these studies have been done on insects collected from the field and reared to the adult stage in the laboratory without knowledge of the initial intake of spores. Other studies have been done on laboratory reared insects, with known doses of spores.

1.1.2.1. Effect on larval development

Infected larvae are not as vigorous and develop more slowly than uninfected ones. Heavily infected larvae may not be able to survive to the adult stage and may die as larvae or pupae. N. pyraustae reduced the survival rate of the European corn borer, P. nubilalis by 21% (Zimmack and Brindley, 1957). Infection of spruce budworm C. fumiferana by N. fumiferanae retarded both larval and pupal development. The mean lengths of larval periods of the infected and non-infected insects were 35.9 and 28.4 days respectively. The lengths of time elapsing between pupation and eclosion were 8.8 and 8 days for infected and non-infected insects respectively, the difference between pupal periods of the

infected and non-infected hosts being statistically significant. As well as retarding development, N. fumiferanae also caused some mortality of C. fumiferana, most of which occurred before the fifth instar (Thomson, 1958a). Larvae of H. zea, which were infected with nuclear and cytoplasmic polyhedrosis virus and N. heliothidis at the same time, died quicker than larvae infected with one pathogen (Lipa, 1968b). Simultaneous infection by nuclear polyhedrosis virus, Pleistophora schubergi and Nosema sp. in larvae of the fall webworm, Hyphantria cunea resulted in lowering of larval mortality. The mortality was 92% for virus infections alone, 21% for Nosema sp. alone and 70% for virus and Nosema sp. together. However larvae which received virus and Nosema sp. treatment together lose their appetite to a greater extent than in the case of infections with virus and Nosema sp. separately (Nordin and Maddox, 1972). Pilley (1976) infected 1st, 2nd, 3rd, 4th and 5th instar larvae of Spodoptera exampta with Vairimorpha necatrix at a dose of 6.75×10^5 spores/larva. She found that susceptibility of larvae decreasd as the age at which they were infected was increased. First instar larvae died 1-2 days after infection, 2nd instars at 3-4 days, 3rd instars at 8-10 days, 4th instars at 8-21 days and 5th instars at 8-24 days after infection. Bacterial infections, which followed penetration of the gut by the microsporidia, occurred in all instars but were less frequent in older larvae.

1.1.2.2. Effect on adult longevity

Adult moths of C. fumiferana infected with N. fumiferanae had shorter lives than non-infected adults. The longevities of infected and non-infected adults were 7.9 and 12.7 days respectively, the difference being significant at the 1% level. The effect was more pronounced among female insects (Thomson, 1958a). N. pyraustae was also found to reduce adult longevity significantly in both males and females of Ostrinia nubilalis. In males it was reduced by 6.45 days from 13.3 days and in females it was reduced by 5.45 days from 12.6 days (Van Denburgh and Burbutis, 1962).

1.1.2.3. Effect on fertility and fecundity

One of the important characters of microsporidian infections is their influence on insect fecundity. This effect has been investigated by several workers, most of them concluding that microsporidia reduce insect fecundity. N. fumiferanae reduced the number of eggs produced by adult females of C. fumiferana by 8 - 9% but there was no evidence that the parasite in male moths affected male fertility, male choice or the fertility of the fertilized eggs (Thomson, 1958a). N. pyraustae significantly lowered the fecundity of O. nubilalis as compared with non-infected females by 47% (Van Denburgh and Burbutis, 1962). Thelohania pristiphorae caused not only a decrease in the number of eggs laid by females of larch sawfly, Pristiphora erichsonii, but also a decrease in the number of ovarioles formed. The average number of eggs varied from 52 to 59 in healthy females and

from 22 to 40 in infected females. Healthy females contained an average of 42.5 ovarioles compared to 39.9 ovarioles for infected ones (Smirnoff and Chu, 1968). Kellen and Lindegren (1971) found that healthy females of Plodia interpunctella, when paired with males infected with Nosema plodiae, laid 792 eggs while females paired with healthy males laid 1350 eggs. They attributed this reduction to damage to the testes caused by the infection. There was also a reduction in viable eggs when diseased females were mated with healthy males (46.3%). This reduction was attributed to the effect of the parasite on ovarian development.

Studies on the pathogenicity of the microsporidian species, which have been used in this study, are reviewed in section 1.4 where the species are considered individually.

1.2 Viability of the free infective stages:

Many studies have been conducted to reveal the effect of sunlight, high temperature and ultraviolet radiation on the viability of spores. Thus, spores in crushed infected midguts were inactivated within 15-32 h after exposure to direct sunlight (White, 1919), while spores of Vairimorpha necatrix tolerated only 5 h of direct sunlight, without loss of infectivity to the armyworm, Pseudaletia unipuncta (Maddox, 1973)., There was a significant decline in infectivity of spores of N. heliothidis extracted from H. zea when exposed to sunlight for up to 4 h. (Sikorowski and Lashomb, 1977). Spores of N. fumiferanae suspended in water and

painted onto cherry leaves became non-infective to the spruce budworm C. fumiferana after only 5 - 6 h exposure to sunlight or 3 - 4 h exposure to a germicidal lamp at a distance of 30 cm (Wilson, 1974). Ultraviolet radiation inactivated spores of Pleistophora schubergi and Nosema agrotidis, pathogens of the turnip moth, Agrotis segetum: infectivity of spores in aqueous suspension decreased from 100% to 10% with increasing power from a germicidal lamp, i.e. from 136.8 to 10,260 erg/cm^2 (Nilova, 1974). Naked spores of Octosporea muscaedomesticae, a pathogen of the black blowfly, Phormia regina were easily inactivated by ultraviolet radiation. The naked spores, when dry, lost their infectivity at a faster rate than naked spores suspended in an aqueous medium (Teetor and Kramer, 1977). However, V. necatrix spores persisted on bean leaves in the field for several days, when an ultraviolet radiation protectant was used (Kaya, 1977).

In temperate zones the viability of most microsporidian spores is greatly reduced by exposure to temperatures higher than 35^{o}C (Maddox, 1973). Humidity plays a certain role in spore viability. As the humidity of the leaf surface is higher than that of the surrounding air (Burrage, 1971), the leaf gives some protection to the spores. V. necatrix survived on bean leaves in the field for 28 h at temperatures ranging from 9 - 26^{o}C with direct exposure to sunlight. Octosporea muscaedomesticae survived 10 months in dried fecal deposits at 5^{o}C and 50% R.H. Drying is harmful for most microsporidian spores. Dried

spores extrude their filaments and lose their viability when they are placed in water (Maddox, 1973). Spores show reduced viability when stored for a prolonged period of time; thus V. necatrix spores were 10,000 times less infective than fresh spores after they were stored in water for 2 years at 5°C (Maddox, 1973). Some spores can withstand temperatures below 0°C. Spores of N. fumiferanae remained infective after 4 months storage in aqueous suspension at -5°C but at 5°C aqueous suspensions of spores of N. fumiferanae lost most of their viability after 4 weeks storage (Thomson, 1958b). Spores are not viable for extended periods even if they are frozen in the cadavers of the host (Thomson, 1958b).

These results demonstrate that spores are unlikely to survive from year to year in the external environment, except N. fumiferanae (4 months frozen). Even these may lose viability when spring comes. When generations of hosts are separated by long periods, as for example overwinter, the spores must either survive in living larvae or adults or by entering the eggs.

1.3 Transmission

Transmission mechanisms fall into two broad categories: a) horizontal when transmission occurs within and between generations of the host but not directly from parent to offspring and (b) vertical - when the parasite passes directly from parent to offspring. Two types of vertical transmission are distinguished: a) transovum transmission

when the pathogen is absorbed on to, or contaminates, the exterior of the egg at the time that the eggs are laid and infection results when the newly hatched host contacts or eats its own egg case, b) transovarial transmission when the pathogen actually gains entry to the egg from the reproductive organs of the parent.

1.3.1. Horizontal transmission

Fine (1981) emphasized that horizontal transmission is the most common method of transmission of parasites in nature. It occurs within and between generations of hosts when generations overlap. Some microsporidia e.g. Nosema locustae and Vairimorpha necatrix are transmitted only horizontally. N. locustae is a highly pathogenic parasite of the fat body of many species of locusts and grasshoppers and a species of cricket. Ewen and Mukerji (1980) studied N. locustae in Melanoplus sanguinipes, M. packaridii and Camnula pellucid, but found no spores in the hosts' eggs. Vairimorpha necatrix has a normal developmental cycle similar to Nosema, producing a mass of spores which are infective per os. It is highly pathogenic to its known hosts, which almost always die before adult so that no vertical transmission can occur. However, at low temperature a second developmental sequence is triggered, producing a second type of spore which is uninucleate and pansporoblastic (i.e. they develop within a pansporoblast membrane in groups of 8 (Maddox, Brooks and Fuxa, 1981 and Malone, 1984)). Malone (1984) produced some evidence that both types of spore are directly infective to

host larvae per os. If this is so, it makes an interesting contrast with dimorphic species in haematophagus Diptera (page 35) in which the pansporoblastic spores are not directly infective to new hosts. Nosema stegomyiae (= N. algerae), a parasite of Anopheles gambiae is another species which is transmitted exclusively by the horizontal route. Fox and Weiser (1959) found that meronts from fat body invaded reproductive tissues, including the terminal filaments of the ovaries, epithelial layer and interstitial tissues but meronts did not invade the follicular epithelium. Heavily infected follicles failed to develop into mature ova and infected trophocytes were destroyed before the maturation of follicles. Heavily infected ovaries were atrophied.

Horizontal transmission thus occurs in multivoltine insects within and between generations and is responsible for wide spread distribution of the parasite in populations. However, in temperate countries these insects have an inactive phase (overwinter) and transovarial transmission would overcome this period of host-dormancy.

In univoltine insects with only one generation per year, horizontal transmission of the parasite is difficult because spores rapidly become non viable on exposure to the environment and vertical transmission is essential for maintenance of the parasite.

1.3.2. Vertical transmission

1.3.2.1. Transovum transmission

Apart from parasitoids, insects lay their eggs in the external environment. Thus when spores contaminate the surface of eggs they are not protected from environmental hazards such as ultravoilet radiation, temperature fluctuation and draught, any more than if they were free. In multivoltine insects when larvae hatch quickly from the eggs, spores will survive long enough for transovarial transmission to occur but when eggs are exposed to environmental conditions for long periods, there is likely to be a rapid fall off in spore infectivity. Transovum transmission has been studied in several insects. Kramer (1959a) found clusters of spores of Nosema pyraustae in the foveae which cover the surface of Ostrina nubilalis eggs. Females of Oncopera alboguttata infected with Pleistophora oncoperae were able to transmit the parasite to 49% of their progeny via the contaminated egg surfaces (Milner and Lutten, 1980). Although males of this moth were not able to transmit the parasite, spores of Nosema fumiferanae from infected males of Choristoneura fumiferana were passed to healthy females in spermatophores, were stored in spermathecae, and contaminated the surface of the eggs (Thomson, 1958b). Spores of a Nosema sp. were found in the spumalin and on the surface of the chorion of Malacosoma americanumm (Nordin, 1975).

1.3.2.2. Transovarial transmission

Transovarial transmission is a more efficient mechanism than transovum transmission because the spores or developmental stages are protected from the detrimental effects of the environment, and are thus offered an effective mechanism for survival over long periods of dormancy. Another advantage is that a high percentage or all the progeny of a single female are infected.

Most stages of microsporidia are capable of transmitting infections by the transovarial route. However, there is evidence, at least in some species, that some stages are transmitted more frequently than others. Spores may be involved but tend to remain in the yolk rather than enter the larval tissues (Thomson, 1958b; Cannin, Barker, Page and Nicholas, 1985). In a few cases mature spores develop from meronts which infect midgut epithelium, fat bodies and hypodermis of the embryo but only single or small groups of cells are infected. When the larvae become active the intensity of infection increases and becomes generalized.

Passage of Nosema bombycis via the eggs of silkworm Bombyx mori was reported by Pasteur (1870) and since that time many other workers have reported transovarial transmission of microsporidian disease.

Zimmack and Brindley (1957) observed N. pyraustae in females of P. nubilalis. The germarium and follicular epithelium were infected and masses of spores occurred in the yolk of many oocytes. Stages were also present within the

oocytes. Kramer (1959a) identified meronts and spores of N. pyraustae among yolk granules of eggs of P. nubilalis.

Nosema plodiae is transmitted within the eggs of Plodia interpunctella as spores or "trophozoites" (Kellen and Lindegren, 1971). Not all stages of N. plodiae have been seen in P. interpunctella with equal frequency: most eggs harboured predominently "trophozoites" (= meronts). Thus mononucleate and binucleate "trophozoites" made up more than 95% of all stages in 80.4% of all eggs. The converse, i.e. spores making up 95% of the total, occurred in only 6.5% of eggs and about equal numbers of both spores and trophozoites were present in the remainder (Kellen and Lindegren, 1973).

Nosema heliothidis is transmitted transovarially in Heliothis zea, a multivoltine insect, which has several generations during spring and summer and enters hibernation as pupae in autumn (Phillips and Barber, 1929; Barber and Dicke, 1937; Barber, 1937 and Phillips and Newson, 1966). Binucleate meronts of N. heliothidis were the predominant forms observed in the infected eggs of H. zea, though a few spores were occasionally detected (Brooks, 1968). Meronts were detected in the developing trophocytes and oocytes within the ovarioles of female but follicular epithelium was completely devoid of infection. The testes of the male H. zea were also heavily infected. Spermatogonia and even mature spermatozoa contained developmental stages of the parasite. The developmental stages were especially conspicuous in the caps of gelatinous material in which the heads

of the bundles of sperm were inserted. Spores were found in the peritoneal and epithelial sheaths. Lipa and Issi (1979) demonstrated the invasion of the follicular epithelium of the ovarioles of Laphygma exigua by N. heliothidis and showed that the primary oocytes, enclosed by follicular epithelium, also harboured several meronts. It was interesting to note that the follicular epithelium infected by N. heliothidis was multilayered due to proliferation. Additionally spores of N. heliothidis were located in epithelial cells, in the trophocytes and also in protuberances made by the trophocytes into the oocytes.

Frozen sections of eggs of eastern tent caterpillars, Malacosoma americanum dissected from virgin females infected with Nosema sp., contained developmental stages (Nordin, 1975). Histological examination of diapausing embryos within overwintering eggs demonstrated that most of the spores occurred in the yolk which filled the lumen of the embryonic fore- and mid-gut regions. Occasionally certain mid-gut cells were observed with light infections. Post-diapausing embryos still contained yolk in the lumen of the fore- and mid-gut. The embryonic tissues, including the mid-gut epithelium, fat body and the hypodermis, were infected but infection was restricted to single or small groups of cells (Nordin, 1975).

N. fumiferanae is restricted to Choristoneura fumiferana a univoltine insect. The insects do not feed from the last larval instar in July until the emergence of 2nd instars from

their hibernacula in May and the spores are unlikely to survive in the environment during this period. Thomson (1958b) provided evidence that the microsporidian parasite, N. fumiferanae, is passed from infected females of C. fumiferana to their offspring via the eggs. Immature eggs within the ovarioles contained meronts. Sections of freshly laid eggs were devoid of spores but meronts could be distinguished among the yolk granules. Spores were seen among yolk granules within the gut of first instar larvae which were killed and fixed immediately after hatching. Eggs, sectioned at the black head stage were found to have spores in the yolk. The parasite was inactive in the yolk mass of the gut, in the first instar larvae and overwintering period of the second instar larvae. Infection of the larval tissues occurred near the time of spring emergence when the spores germinated.

Canning, Wigley and Barker (1983) studied three species of microsporidia in winter moth, Operophtera brumata, and found that the predominant stages in eggs varied according to species. Pleistophora operophterae was present mostly as free spores. Only a few sporophorous vesicles were found and meronts and multinucleate sporonts were not seen. Orthosoma operophterae occurred mainly as sporogonic stages with a few meronts. Nosema wistmansi occurred only as binucleate meronts. Canning, Barker, Page and Nicholas (1985) followed the development of O. operophterae during embryonation of the winter moth eggs and found that the microsporidia were

restricted to the yolk. They were present in yolk material between the serosa and the larva and in the meconium in the lumen of the gut. It was thought that, as the larva started to eat its way through the egg shell and the digestive tract became functional, the spores were ingested and infected the larva via the epithelial cells of the digestive system.

1.3.3. Transmission of the dimorphic microsporidia

A large group of microsporidia, involving several closely related genera, have two completely different sequences of sporogony, giving rise to recognizably different types of spores. Sometimes the two types of spores occur alongside one another in the same tissue, sometimes they develop in different tissues and sometimes they are produced at different stages of host's life cycle. The two spore types appear to perform different roles in transmission. Many of the dimorphic microsporidia parasitise haematophagus Diptera. Examples are found amongst the species of Amblyospora and Parathelohania parasitic in mosquitoes. In one cycle, uninucleate haploid spores are produced principally in male larvae but these spores are not directly infective to mosquitoes and their role in transmission is unknown. In the other cycle, binuleate spores are produced in adult female mosquitoes and these are responsible for transmission of the infection via the eggs to the next generation of larvae. Although vertical transmission is the only known method of transmission of these microsporidia, there must be an element of horizontal transmission, as the progeny of the uninfected

females have a survival advantage over those of the infected. Dimorphism was first reported by Hazard and Weiser (1968) who described the developmental stages of Thelohania (= Parathelohania) legeri and T. (= P.) obesa in males and females of Anopheles . In male larvae there were vegetative stages with 1, 2, 4 or 8 nuclei that produced pansporoblasts contaning 8 spores. In female larvae there were only dormant sporonts containing 1, 2 or 4 nuclei. These were in the haemolymph and the infections did not usually produce spores. In adult females the parasites were first seen as multinucleate sporogonial plasmodia containing 4 - 32 nuclei. After the first blood meal, the plasmodia divided into many binucleate cells, some of which invaded the ovaries, while others formed cylindrical spores in the fluid between the ovarioles. The progeny of these females carried the infection.

Kellen and Wills (1962) studied the stages of Thelohania californica in mosquitoes. This parasite is clearly related to those seen by Hazard and Weiser (1968) but the stages in adult females, responsible for transovarial transmission were not recognized. Kellen and Wills found that eggs laid by Culex tarsalis infected with T. californica, contained mono- and binucleate meronts. Male larvae from infected eggs were heavily infected, with sporonts and spores and usually died, due to the infection, during the 4th larval instar, while female larvae were infected only with meronts. Sporogony did not occur in female larvae; infected female larvae pup-

ated normally and emerged as apparently healthy adults but when mated with healthy males, laid eggs containing meronts.

Andreadis (1983) investigated the transovarial transmission of Amblyospora sp. in Aedes cantator. Like Hazard and Weiser (1968), he found that Amblyospora sp. had two quite different developmental sequences. Infections were initiated in adult females when small sporoplasms were released from binucleate spores contained within host oenocytes. The sporoplasms entered the developing oocytes and were subsequently transmitted to the filial generation. In a proportion of female progeny, hatching from these eggs, diplokaryotic cells were observed within host oenocytes. Here they entered a latent phase of development, during which very little multiplication occurred. Female larvae with such infections showed no visible symptoms of disease, pupated normally and emerged as healthy adults. However, shortly after adult emergence, the microsporidia entered an accelerated phase of merogonic division during which diplokaryotic stages divided by binary fission and caused host oenocytes to become hypertrophied. Sporulation did not occur until a blood meal was taken by the female host, so that spore production coincided with the development and maturation of the host oocytes, which then became infected with sporoplasms. The second developmental sequence occurred in other female and all male progeny. Development occurred within fat tissue of the host where diplokaryotic cells divided by multiple fission during merogony to spread

the infection. Sporulation in this developmental sequence was characterized by development of the sporont in a pansporoblast membrane and meiotic division in the early diplokaryotic sporonts. These processes resulted in the formation of octonucleate sporonts with isolated nuclei and, after cytokinesis, eight haploid spores were formed, which were not orally infective to other mosquito larvae. In these life cycles no direct horizontal transmission of the pathogen has been found.

Dimorphism is also known to occur in Burenella dimorpha a microsporidian of tropical fire ant, Solenopsis geminata (Jouvenaz and Hazard, 1978). There are two sequences of sporogony; one sequence has disporous sporonts that sporulate in the hypodermis, producing binucleate non-membrane bound spores (ie. not in a pansporoblast membrane) which are infective per os and the other sequence has multinucleate sporonts that produce 8 uninucleate spores in a pansporoblast membrane, which infect fat tissue and are not transmitted orally. The two sequences closely resemble those of Amblyospora and Parathelohania in mosquitoes but occur in different tissues at the same stage in the life cycle of the host.

Vairimorpha necatrix has temperature-dependent dimorphism so that at 25^{o}C a predominant disporoblastic sequence produces free binucleate spores and at 20^{o}C or below an octosporoblastic sequence produces 8 uninucleate spores contained in a pansporoblast membrane. There is limited

evidence that octospora are directly infective to new hosts (Malone, 1984) (see also page 28)

1.4 Life cycles of the microsporidia used in this study

1.4.1 Nosema heliothidis

1.4.1.1 Nomenclature

Nosema heliothidis Lutz and Splendore, 1904 was isolated from Heliothis zea in Brazil (Lutz and Splendore, 1904). The original spelling N. heliotidis was amended by Steinhaus (1949), who considered that the omission of the second "h" was unintentional as the parasite was clearly named after the generic name of the host.

1.4.1.2 Life cycle

Kramer (1959b) described the principal stages of the developmental cycle. The smallest meronts of the parasite are usually rounded, measuring 3.0 - 4.5 µm in diameter and contain two deeply staining nuclei. Tetra nucleate meronts measure up to 7.5 x 11.5 µm and they undergo binary fission into two binucleate daughter meronts. In sporogony binucleate meronts develop into a sporont, which in turn produces two sporoblasts which mature into spores. Binucleate sporonts are usually elongate to elliptical and measure 3.0 - 3.5 x 5.4 - 7.0 µm. The typical early sporoblast is ovoidal in form, measuring 3.5 x 5.5 - 7.0 µm. Fixed and stained spores measure 2.5 - 5.5 µm long x 2.0 - 3.5 µm wide at the point of their greatest breadth. Extended polar filaments range from 105 - 115 µm in length.

1.4.1.3 Pathogenicity

Lipa (1968a) and Brooks and Cranford (1978) demonstrated the histopathological effect of N. heliothidis in H. zea. Diseased larvae are not as active as healthy ones and they do not feed readily. A high percentage of infected larvae fail to pupate or produce abnormal pupae. Normal tissues are translucent, while infected organs are opaque or whitish in colour, infected haemocytes become hypertrophic then degenerate and are destroyed. Muscles are only mildly infected but the fat body is heavily infected. The midgut epithelium is particularly heavily infected and in advanced infections almost all eipthelial cells are destroyed. The main site of infection in adults is the fat body but the parasite may also be observed in the ovaries and testes.

Gaugler and Brooks (1975) compared the reproductive and diapause potentials of H. zea infected with N. heliothidis with those of uninfected individuals. Whether transmission took place per os or transovarially, the reproductive potential was significantly affected due to reductions in fecundity, longevity and mating success. Under laboratory conditions 13% fewer infected pupae entered diapause and the duration of the period of pupal diapause was reduced by 10 - 6 days. Larval development was retarded by 3 days and there were slight increases in the incidence of pupal deformaties and mating pairs which failed to separate. Egg hatching and direct host mortality were not influenced adversely by parasitisation.

Brooks and Cranford (1972) and Brooks (1973) studied the interaction of H. zea infected with N. heliothidis and the ichneumonid larval parasitoid Campoletis sonorensis. The parasitic larvae ingest host tissue during their development and on emergence, cellular debris and spores of the microsporidium fill the midgut lumen of the larval or pupal stage of the parasitic insect. The larval, pupal and adult stages of C. sonorensis carry infections of N. heliothidis, which are particularly heavy in the larval midgut epithelium and in adult reproductive tissues. Spores of N. heliothidis infect the transparent eggs of female parasitoids and the microsporidium is transmitted by C. sonorensis to H. zea.

McNeil and Brooks (1974)studied the interactions of the hyperparasitoids (parasites of parasitoid insects) Catolaccus aeneoviridis and Spilochalcis side with N. heliothidis and another species, Nosema campoletidis and found that neither of the hyperparasitoids was susceptible to infection by N. heliothidis and that there was no evidence of nutritional imbalance due to accumulation of spores in the midgut of hyperparasitoid larvae (C. aeneoviridis and S. side).

Brooks and Cranford (1978) also showed that N. heliothidis is highly prevalent in natural populations of H. zea and the rate of transovarial transmission is almost 100%. Under laboratory conditions, only a few species of noctuids and one hymenopterous parasitoid have been found to be susceptible to infection. N. heliothidis has only a sublethal effect in H. zea. It reduces adult longevity, female fecund-

ity and mating ability of adults. Midgut and silk glands are the main sites of infection. Infection is less extensive in the fat body and is characterized by an extensive inflammatory response. In contrast, in the fat body of adults, there is no marked inflammatory response. Infection in H. virescens is more limited and is conspicuously marked by inflammation.

Brooks (1968) detected schizogonic stages of N. heliothidis in developing trophocytes and oocytes of H. zea. Also the testes of males were heavily infected.

1.4.2. Nosema bombycis

1.4.2.1 Nomenclature

N. bombycis, the causative organism of pebrine disease in the silk worm, Bombyx mori was discovered and named by Pasteur (1870).

1.4.2.2 Life cycle

N. bombycis is now known to have a wide host range largely within the Lepidoptera (Sprague and Vavra, 1977). In 1870 Pasteur reported the passage of N. bombycis through the eggs of B. mori. Since that time transovarial transmission has been recognized as the principal mean of spreading the disease among the insect culture.

Spores are oval or pyriform in shape and measure 3 - 4 µm long x 1.5 - 2 µm wide. Nearly all stages of the silk worm are invaded (Steinhaus, 1949).

The fine structure of N. bombycis developing in the midgut epithelium of B. mori was investigated by Ishihara

(1970). N. bombycis typically has nuclei in diplokaryotic arrangement, although stages with single nuclei were seen. The meronts increased in size during the first 24 h after inoculation then divided. Transformation into spores started around 48 h after inoculation. It was indicated that sporulation stages were distinguished from meronts by the appearance of additional cisternae of rough surface endoplasmic reticulum. Cali (1970) confirmed the diplokaryotic nature of all stages.

1.4.2.3 The development of N. bombycis in tissue culture

Ishihara and Sohi (1966) used an ovarian tissue culture of B. mori to study the developmental stages of N. bombycis. They found that some young meronts had "tails". Lanceolate binucleate meronts developed from young binucleate meronts 24 h after inoculation and the sporoblasts appeared 72 h after inoculation. Spores filled host cells 21 days after inoculation and infected cells increased in size and nuclei were hypertrophic. In 1969 Ishihara further studied the life cycle of N. bombycis in cultured cells of B. mori and identified as sporoplasms binucleate stages seen in cells shortly after the spores had been added to the cells. During the first few hours after inoculation, the microsporidia increased in size while keeping the original round appearance, they then changed to an elongated form before undergoing binary fission. Elongated forms increased in number by binary fission until finally sporonts were produced each of which gave rise to two spores. A round form possessing

two nuclei and compact cytoplasm was thought to be responsible for spreading the infection from one cell to another within the host and was termed the secondary infective form. Kawarabata and Ishihara (1984) recognized 3 successive stages in the development of N. bombycis in Antheraea eucalypti cells. In the first stage the sporoplasm or the secondary infective form grew into a meront. This was followed by a proliferative stage, during which there was repeated multiplication by binary fission of the meronts. Finally, there was a stage of differentiation during which the sporont gave rise to two sporoblasts then spores. The secondary infective forms were produced during the early stages of sporoblast development, and the infective form invaded the host cell immediately after its release into the culture medium. The secondary infective forms are able to emerge from a host cell and invade a new host cell by themselves.

1.4.2.4 Pathogenicity

Heavily infected organs are hypertrophic and have a milky-white appearance. Brown or black spots appear on the cuticular surface of the infected larvae. Females of Bombyx mori infected with N. bombycis had a lower average fecundity, which was dependent both on spore dosage and on larval instar in which infection was initiated. Veber and Jasic (1961) found that the mean fecundity of healthy females of B. mori was 453 compared to 341 in females infected at 5th instar with a dose 5×10^3 spores/larva. The mean number of eggs laid per female was reduced to 322 by exposing the 5th larval

instar to a dose of 5×10^4 spores/larva while an even greater reduction in fecundity was obtained when larvae were infected at the beginning of the 4th instar with 5×10^3 spores/larva.

1.4.3 Nosema locustae

Canning (1953) isolated a new microsporidium, which she named Nosema locustae from the fat body of the African migratory locust, Locusta migratoria migratorioides. Following the initial description of N. locustae, knowledge of the host range was extended to include the desert locust, Schistocerca gregaria, and five species of grasshoppers from North America, including the economically important Melanoplus sanguinipes, Melanoplus bivittatus and Camnula pellucida (Canning, 1962a). Henry (1969) reported that 58 species of grasshoppers, a species of cricket and a pygmy locust were also susceptible to infection.

1.4.3.1 Life cycle

In 1962, Canning described the development of N. locustae. Binucleate meronts, measuring 3 - 4 µm became tetranucleate meronts, measuring up to 5 or 6.5 µm. Reproduction continued inside the host cell until it became packed with rounded products of merogony and ovoid stages which were interpreted as sporonts. Today these would be considered as sporoblasts, the product of division of the sporont. These stages became ovoid and developed a thick wall. Mature spores measured 4-6.5 µm x 2.6 - 3.5 µm. Huger (1960) studied the ultrastructure of the spore of N.

locustae. Although this was the first attempt at electron microscopy of microsporidia, which were notoriously difficult to fix, some details were revealed. The spore showed a smooth surface with a wall consisting of a thick inner electron-lucent spore coat up to 380 nm thick and a thin electron dense coat 30 - 45 nm thick. The polaroplast was revealed as a lamellar structure and the remainder of the intrasporal space was occupied by the sporoplasm with two nuclei and the polar filament (polar tube). The polar tube showed up to eighteen turns.

1.4.3.2 Pathogenicity

The main site of infection was the fat body which suffered great hypertrophy and conversion of the normal bright yellow colour to an opaque, creamy mass. The parasite was thought to invade the fat body exclusively, so that the reproductive organs, accessory glands and Malpighian tubules were not infected (Canning, 1953). Henry (1967) confirmed the absence of stages of N. locustae from the digestive system and gonads of grasshoppers. During an investigation to test whether N. locustae could be used for control of grasshoppers, Henry (1971b) observed that grasshoppers exhibiting light or heavy levels of infections rarely possessed detectable quantities of ovarial tissues or egg debris, which suggested to him that they lacked normal reproductive capacity. In the laboratory, nymphs infected during the 3rd instar or earlier developed slowly and usually died during the 4th or 5th instar. However, nymphs infected

during the 4th and 5th instars usually developed and persisted for prolonged periods as adults. In field trials using spores spread on a bran bait Henry, Tiahrt and Oma (1973) demonstrated that the concentration of N. locustae spores was the most important factor in reducing the densities of grasshoppers and the incidence of infection among the survivors, rather than timing and levels of spore carriers in the applications. The average reduction attributed to 1 spore/in^2 was 21%, that attributed to 3 spores/in^2 was 46% and that attributed to 900 spores/in^2 was 73%. Higher prevalence of infection were observed among grasshoppers treated with 30 spores/in^2 and 1 lb bran/acre than 30 spores/in^2 in 4 lb bran/acre.

Ewen and Mukerji (1980) saw no spores or sporonts within the eggs or the froth surrounding the egg pods of grasshoppers Melanoplus sanguinipes, M. packaridii and Camnula pellucida treated with N. locustae. Some of the eggs were hatched and the grasshoppers were examined after they had moulted to at least the 3rd nymphal instar. In these, trace levels of infection were found in about 10% of the insects of each of the 3 species from the treated plots.

1.4.4 Pleistophora operophterae

P. operophterae was described in Operophtera brumata by Canning, (1960).

1.4.4.1 Life cycle

Uninucleate meronts measured 3.25 x 2.5 µm. Multinucleate plasmodia gave rise to 10 to 100 sporoblasts

measuring 3.1 x 1.8 µm produced within pansporoblast membranes (sporophorous vesicles). The parasite was redescribed by Canning, Wigley and Barker (1983). There were plasmodia with varying numbers of nuclei, some of which were meronts with dense homogenous cytoplasm and low number of nuclei. Meronts were capable of division by multiple fission. Sporonts were difficult to differentiate from meronts except by the greater number of nuclei. The uninucleate, pale-staining sporoblasts were loosely arranged within sporophorous vesicles but on maturation into spores the sporophorous vesicles appeared tightly packed. The number of spores derived from the sporogonial plasmodia was thought to range from 4 to 60 with 8, 12 and 16 the most common numbers. Fresh spores were elongate ellipsoid, without visible posterior vacuole. Measurements of 2.4 $\pm$ 0.6 x 1.2 $\pm$ 0.2 µm were given for stained spores. Fresh spores measured 2.5 $\pm$ 0.2 x 1.2 $\pm$ 0.1 µm and fresh sporophorous vesicles ranged from about 4.0 to 12.0 µ diameter. In an ultrastructural study Canning, Barker, Nicholas and Page (personal communication), have shown that nuclei are isolated. All stages develop in a vesicle bounded by an envelope of enigamtic origin. This envelope persists around the spores as a sporophorous vesicles. Division of the sporont within this vesicle occurs by budding and the number of sporoblasts, and therefore spores is variable up to 60.

Stages of P. operophterae in smears of non-embryonated eggs were mostly free spores. Vegetative stages were not

seen and sporophorous vesicles were rare and large. Fusiform bodies with varying stained cytoplasm and polar regions were also in smears of non embryonated eggs.

1.4.4.2 Pathogenicity

P. operophterae is a parasite of the salivary glands, gut, fat body, muscles and Malpighian tubules of larvae, pupae and adults. It invaded the cells of silk glands in a very regular manner and the cells were only slightly hypertrophic and the lumen of the glands was unobstructed.

1.5 The female reproductive system of insects

The female genital system consists of 2 ovaries, one on each side, and accessory glands. The ovary consists of ovarioles, the number of which varies greatly with species. Bonhag (1958) and Rockstein (1973) recognised 3 main regions in the ovariole:

1. Germarium, in which oogonia are formed from germ cells and are enveloped by mesodermal cells. At the base of the germarium, there is prefollicular tissue which is formed from somatic mesodermal cells.
2. Vitellarium, a long region and its length depends on the reproductive activity of the insect. It contains a series of developing oocytes, which are always arranged in a single row. They are enveloped in an epithelial follicle and they increase in size as they reach to the end of the vitellarium.
3. Ovarial stalk, where the fully grown eggs are stored, before they pass to the oviduct. Bonhag(1958) recognized two main types of ovarioles in insects, which he named

panoistic and meroistic ovarioles. In panoistic ovarioles there are no trophocytes and the follicular epithelium is the only trophic tissue present. Meroistic ovarioles have trophocytes which are associated with oocytes. Meroistic ovarioles are divided into two subtypes, polytrophic and telotrophic ovarioles. In polytrophic ovarioles trophocytes alternate with oocytes in the vitellarium, but in telotrophic ovarioles, trophocytes are concentrated in the germarium and are lined with the oocytes by cytoplasmic cord.

1.5.1 Panoistic ovary

Panoistic ovarioles are present in the orders Thysanura, Orthoptera, Isoptera, Odonata and Plecoptera. Each ovarioles is covered by an outer epithelial sheath and follicular epithelium and between these layers is a non-cellular layer known as the tunica. Each ovariole consists of a terminal filament, germarium region and vitellarium. The terminal filament is a syncytial strand that performs a suspensory function. The germarium contains numerous oogona, with occasional oogonial cells undergoing mitosis, and young oocytes. The vitellarium consists f fully developed primary oocytes (Figs.1,2). Ovaries of Locusta migratoria, Oxycatantops praemonatus, Oxycatantops annulipes and Catantops sylvestrii are panoistic types.

1.5.2. Meroistic ovary

1.5.2.1 Telotrophic ovary

Telotrophic ovarioles occur in all Hemiptera and many of Coleoptera. Two sheaths cover the ovariole, an outer

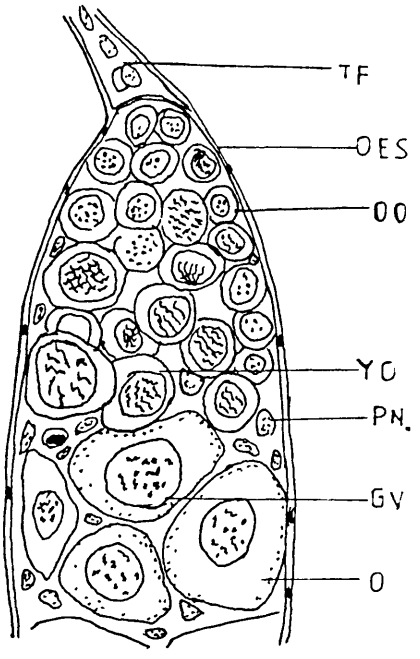


FIG.1

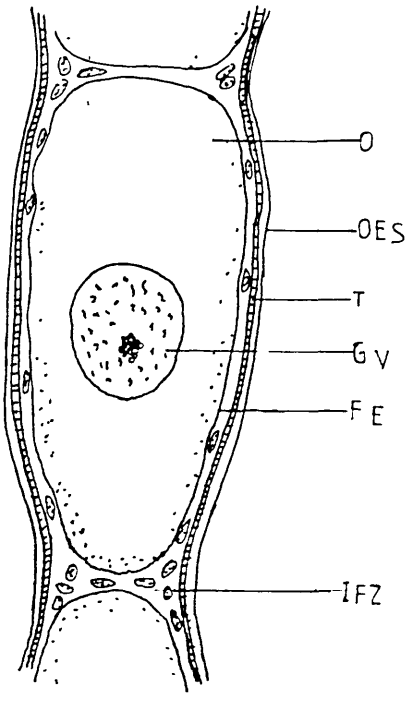


FIG. 2

Fig.1 - Anterior end of panoistic ovariole (From Bonhag, 1958)

Fig.2 - Posterior end of Panoistic ovariole (From Bonhag, 1958).

FE, follicular epithelium; GV, germinal vesicle;

IFZ, interfollicular zone; O, oocyte;

OES, outer epithelial sheath; OO, oogonium;

PN, prefollicular nucleus; TF, terminal filament;

T, tunica; YO, young oocyte

epithelial sheath and an inner envelope. Each ovariole consists of 4 regions: terminal filament, germarium, vitellarium and ovarial stalk. The long terminal filament is bulbous at its posterior end. Apical trophic tissue is the main content of the germarium which can be divided into 3 main zones according to the stages of differentiation of functional nurse tissue (trophocytes)(Fig.3). In the 1st zone the cell boundaries are distinct and the zone is characterized by extensive mitotic activity. In the 2nd zone the cell boundaries have disappeared and trohpic nuclei have moved together to form small clusters of nuclei, embedded in common cytoplasm. The nuclei of zone 3 are arranged in the form of aggregates. The nuclear aggregates are arranged around a central cylinder of cytoplasm known as the trophic core. The trophic core carries at its posterior end a number of projections known as nutritive cords, which extend to the developing oocytes in vitellarium. These cords are the connections through which the nutritive substances from the apical nurse tissue passes to the oocytes.

A mass of prefollicular tissue lies posterior to the apical nurse tissue. There are a great number of oocytes in the prefollicular tissue. As the enlarged oocytes reach the vitellarium, the prefollicular tissue is oriented around them and young follicles are surrounded by columnar epithelium. As the follicles increase in size, the follicular tissue transforms into large binucleate rounded cells and finally

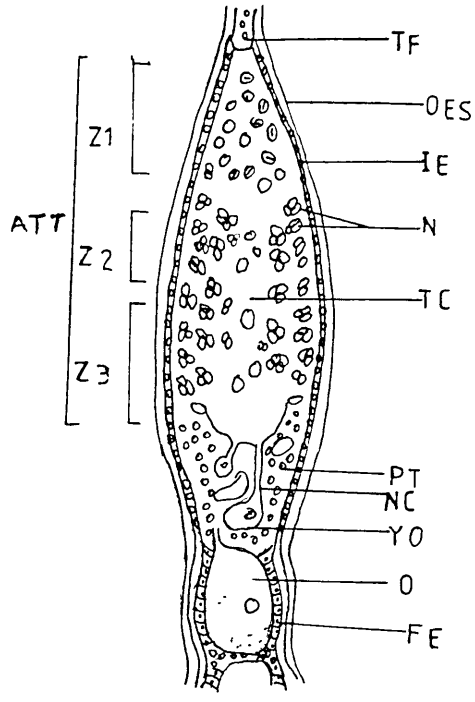


FIG.3

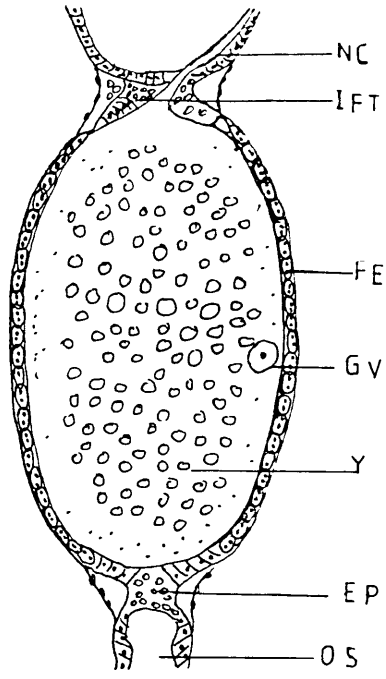


FIG.4

Fig. 3 - Anterior end of telotrophic ovariole

(From Bonhag, 1958)

Fig. 4 - Nearly mature follicle in telotrophic ovariole

(From Bonhag, 1958)

ATT, apical trophic tissue; Ep, epithelial plug; FE, follicular epithelium; GV, germinal vesicle; IF, inner envelope; IFT, interfollicular tissue; N, nuclei; NC, nutritive cord; O, oocyte; OES, outer epithelial sheath; OS, ovarial stalk; PT, prefollicular tissue; TC, trophic core; TE, terminal filament; Y, yolk; YO, young oocyte; Z, zone.

becomes squamous. Each mature primary oocyte, still attached to the nutritive cord, contains a large quantity of yolk and its follicular epithelium, composed of binucleated cells (Fig.4). Follicles are separated from each other by interfollicular tissue. In this system of ovarioles the vitellarium is terminated by an epithelial plug, which breaks down at the time of oviposition to enable mature oocytes to pass into the ovarial stalk.

1.5.2.2 Polytrophic ovary

Most of the holometabolous orders, ie. those in which the life cycle comprises eggs, larvae, pupae and adults, contain insects with polytrophic ovarioles. The wall of each ovariole consists of a syncytial epithelial sheath. Each ovariole consists of terminal filament, germarium, vitellarium and ovarial stalk. The wall of the terminal filament is thin and non-cellular and contains undifferentiated cells, irregularly arranged. The germarium is composed of cells derived from the primordial germ cells. These cells are distinguished as two kinds according to their future development: those destined to become eggs and those which will differentiate into trophocytes. In the distal end of the germarium it is difficult to distinguish between these two components. They all have spherical nuclei surrounded by a small amount of cytoplasm. In the vitellarium region, each oocyte and its related trophocytes line up in a single row to form the follicle. In the follicle, the oocyte always lies posterior to its related

trophocytes. Each follicle is surrounded by follicular epithelium. As the follicle develops and increases in size the follicular epithelium becomes columner, then cuboidal and finally flattened. In the young follicle the oocyte is much smaller than the accompanying trophocytes (Fig.5). In middle-age follicles the oocyte and trophocyte increase tremendously in size and the oocyte becomes slightly greater in volume than the trophocyte (Fig.6). The trophocytes then diminish and start to be absorbed by the oocyte (Fig.7). Finally the trophocytes undergo complete dissolution and the oocyte is released from the vitellarium into the ovarial stalk (Fig.8).

Ovaries of Heliothis zea are polytrophic type.

1.6 The male reproductive system of insects

The testis consists of a number of sperm tubules. The number of the tubules and their arrangement varies in different insects. Each tubule is enclosed in a layer of epithelium, resting on a basement membrane. The entire group of tubules is surrounded by a connective tissue sheath known as the peritoneal sheath. In Lepidoptera the sperm tubules are incompletely separated. The 2 testes are bounded together within a single capsule. Each sperm tubule contains a series of zones which represent the developmental stages of the sperm (Fig.9):

1. Spermatogonia: this zone contains primordial germ cells lying among somatic mesodermal cells.

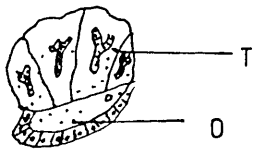


FIG.5

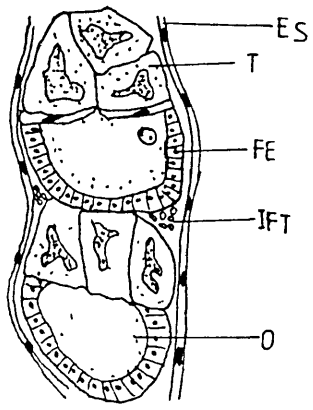


FIG.6

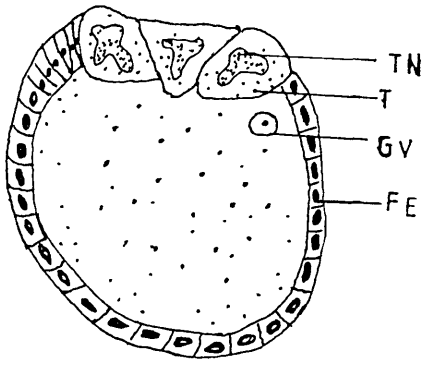


FIG.7

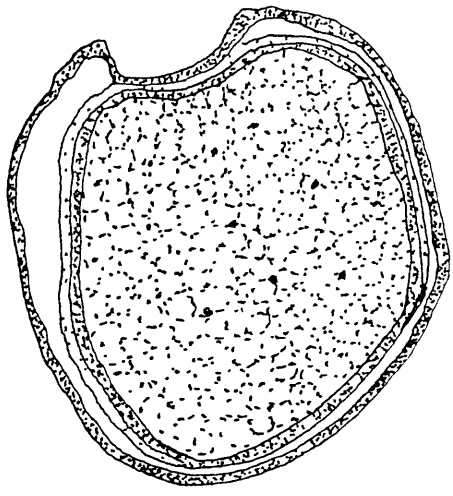


FIG.8

Fig.5: Early stage of development of oocyte.

Fig.6: Middle stage of oocyte development.

Fig.7: Oocyte dwarfs the trophocytes.

Fig.8: Mature oocyte with chorion.

IFT interfollicular tissue; ES epithelial sheath; FE follicular epithelium; GV germinal vesicle; O oocyte; T trophocyte; TN trophocyte nucleus.

2. Spermatocyst: in this zone, spermatogonia divide repeatedly to form cysts.

3. Spermatids: each spermatocyst divides twice to form 4 spermatids.

The first division, in most insects, is a reduction division, in which the number of chromosomes is halved.

4. Spermatozoa: where the spermatids are transformed into spermatozoa.

The primorida of the male reproductive system can also be detected in late larval instars.

In lepidopteran larvae the genital system consists of 2 testes, each divided into 4 sperm tubules. Sperm tubules are separated from each other by folds of epithelial sheath, which extend posteriorly towards the origin of the vas deferens. The entire organ is surrounded by a peritoneal sheath. The testicular tubules (Fig.10) are characterized by the presence of a large cell or nucleated mass of protoplasm in the apex of the germarium. This cell is known as the apical cell. The apical cell is surrounded by several concentric rows of spermatogonia. Spermatogonia undergo a series of divisions to form spermatozoa. Each spermatogonial group is enclosed by a sperm cyst which is a cellular envelope.

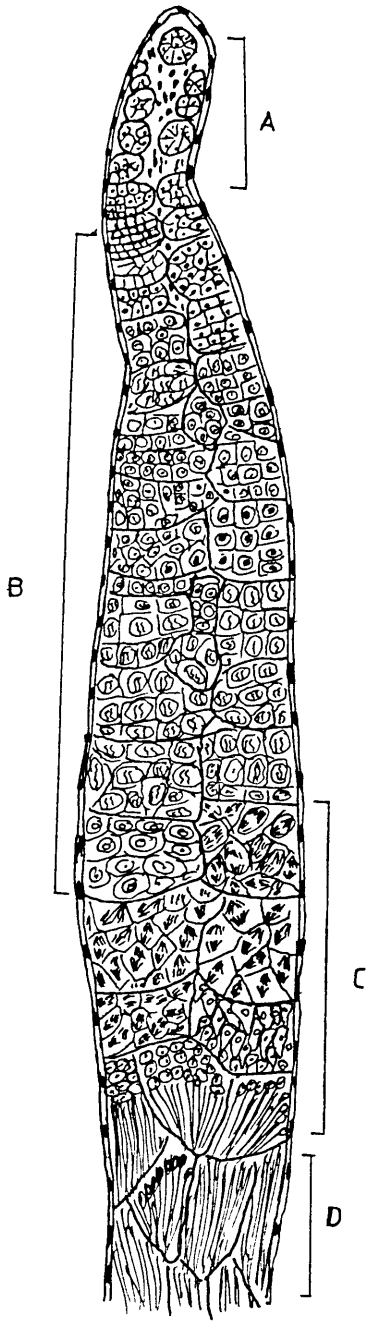


FIG.9

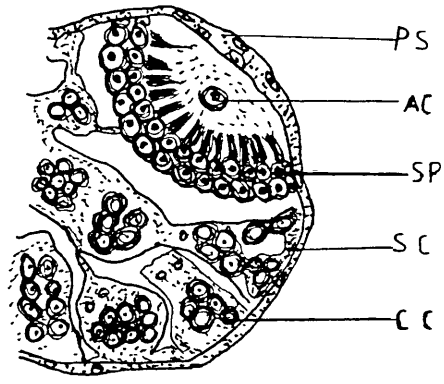


FIG.10

Fig.9 - Sperm tubule (From Wigglesworth, 1947).

Fig.10- Section of a larval sperm tubule (From Snodgrass, 1935).

A, zone of spermatogonia; B, zone of spermatocyst; C, zone of spermatids; D, zone of spermatozoa; AC, apical cell; CC, cyst cell; PS, peritoneal sheath; SC, sperm cyst; SP, spermatogonia.

2 MATERIALS AND METHODS

2.1 Maintenance of insect cultures.

2.1.1 *Heliothis zea*

Eggs of *H. zea*, received from the Institute of Virology, Oxford, were surface sterilized in 0.2% sodium hypochlorite for 5 min, collected on filter paper and washed thoroughly in tap water. They were rinsed in 10% formalin solution, containing a drop of wetting agent Triton X100 for 40 min, collected as before and washed to remove all traces of formalin. They were dried in air, then incubated at 25°C, 70% relative humidity and 12 h light, 12 h dark photoperiod. Since *H. zea* is a restricted pest in the United Kingdom all procedures involving these insects were carried out under quarantine conditions with licences PHF 29/160 and PHF 29/267, from the Ministry of Agriculture, Fisheries and Food, Eagle House, 90-96 Cannon Street, London. Newly hatched larvae were transferred with a paint brush onto pieces of artificial diet (Appendix 1) in small plastic pots (Cristaseal No.10 polypots) at a density of 1 larva/pot. Larvae were incubated at 25°C and additional diet was supplied as required. After the second moult, larvae were prepared for infection by starving for 24 h.

2.1.2 *Locusta migratoria*

Second instar nymphs were obtained from Imperial College at Silwood Park. They were reared in small cylindrical cages, each 9 inches in diameter and 16 inches deep, consisting of base and lid made of polystyrene with a clear

plastic cylinder in between (Watkins and Doncaster Ltd., Strand, London). Each cage contained up to 50 nymphs. The adults were reared in a metal cage measuring 15 x 15 x 20 in, fitted with a glass panel in front for observation purposes and with a roof that could be removed to facilitate periodic cleaning. There was a well fitted removable false floor of perforated zinc, inserted a few inches above the main floor. The cages were incubated in a controlled temperature room at 30°C. Adult cages were provided with a 60 watt electric light bulb for extra heat. Dry branched twigs were stood in the cage to enable the hoppers to moult and rest, and each day a plentiful supply of fresh grass and dry wheat bran was placed in the cages. The cages were cleaned daily, removing any uneaten grass from the previous day together with faeces and dead locusts, before fresh grass was added. Metal cages were provided with egg-laying tubes 1.5 inches in diameter and 4 inches deep, filled with clean sterilized sand moistened with 15% distilled water. They were inserted between the false and main floors of the cage and renewed every 2 or 3 days whether or not eggs had been laid. The tubes were covered with a loose fitting metal cap to prevent excessive evaporation of water and incubated at 28 - 32°C. The tubes were not disturbed until a day or two before the eggs were due to hatch, then the covers were removed and the tubes placed in the cages in which the resulting hoppers were to be reared.

2.1.3 Oxycatantops praemonatus, Oxycatantops annulipes and Catantops sylvestrii

Eggs of O. praemonatus, O. annulipes and C. sylvestrii were obtained as eggs in sand-filled tubes from the Tropical Development Research Institute, College House, Wrights Lane, London. The egg-laying tubes were incubated at 30-32°C and a few drops of distilled water were added every 3 days to prevent the sand from drying out. The caps of the tubes were removed 2 days before the eggs were due to hatch. The tubes were kept in a controlled temperature room at 30°C inside lamp glass containers measuring 2.5 inches in diameter and 13 inches deep. One end of each container was covered by muslin, held on by a rubber band and a petri dish lined with filter paper was used as a base. After hatching the hoppers were supplied daily with fresh Chinese cabbage leaves as a source of food. After first moult, the grasshoppers were transferred to cylindrical cages as for the locust hoppers, each cage containing up to 80 grasshoppers and fresh grass was given instead of Chinese cabbage leaves. The cages were cleaned daily, removing any uneaten grass from the previous day together with faeces and dead grasshoppers, before new grass was added.

2.2 Preparation of spores

2.2.1 Nosema heliothidis

A stock suspension of spores was prepared from infected pupae of Heliothis zea obtained from Dr.W.M.Brooks, Department of Entomology, North Carolina State University.

The infected pupae were homogenized in sterile water in a tissue grinder. The homogenate was strained through a double thickness of muslin and the filtrate was centrifuged for 15 min at 750 g. The sediment was resuspended in distilled water and the concentration of spores was estimated by counting with an improved Neubauer haemocytometer. Spores were stored at 4°C in sterile plastic 30 ml universal containers.

2.2.2 Nosema bombycis

A spore suspension of N. bombycis was obtained initially from Dr.G.G.Wilson, Canadian Forestry Service, Forest Pest Management Institute, Canada. The spore suspension was used to infect 3rd larval instar of H. zea. Spores for experimentation were harvested from these larvae and pupae. Dead bodies were homogenized in distilled water in a tissue grinder. The homogenate was treated as for N. heliothidis.

2.2.3 Nosema locustae

A suspension of spores of N. locustae was obtained from Dr.J.E.Henry, Rangeland Insect Laboratory, Federal Research Science and Education Administration, U.S. Department of Agriculture, Bozeman, Montana. The suspension was used to infect 3rd instar nymphs of Locusta migratoria which were reared to adult stage. Abdomens of infected adults were dissected to obtain infected fat tissues which were homogenized in distilled water in a tissue grinder. The homogenate was treated as for N. heliothidis.

2.2.4 Pleistophora operophterae

Spores of P. operophterae, which were used in these experiments, had been harvested from experimentally infected Operophtera brumata.

2.3 Administration of spores

2.3.1 Administration of spores to Heliothis zea

Doses of 1.0×10^6, 3.2×10^6 and 4.4×10^6 spores of Nosema heliothidis per larva were used in 3 separate experiments to infect groups of 50 3rd instar larvae (at each dose). A control group of 150 larvae were reared spore free.

The doses for N. bombycis were 6×10^3, 2×10^4 and 4×10^5 for separate groups of 30 2nd instar larvae and 4×10, 4.8×10^2 and 6×10^3 for separate groups of 30 5th instar larvae. A control group of 180 larvae were reared spore free.

Doses of 2×10^4, 2×10^5 and 2×10^6 spores of Pleistophora operophterae per larva were used to infect 90 2nd instar larvae, 30 larvae for each dose, and a control group of 90 larvae were reared spore free.

Larvae were starved overnight then transferred individually to pots in each of which was placed a 3 mm diameter disc of semisynthetic diet (Appendix 1) to which the spores had been applied in 2 µl distilled water by micro pipette (Pipetman P20). When the whole disc had been consumed, each larva was transferred to a 3 cm pot containing a layer of diet. Frass was removed daily from the pots and

larvae were transferred to fresh pots when the diet was consumed or fungal growth was observed.

After 10 days of treatment, 2 larvae (for each dose) from each test and control group were removed and checked for infection by examining wet mounts or stained smears of guts and additionally the salivary glands for the P. operophterae infection with a Leitz Dialux 20EB microscope, by phase contrast microscopy at 250x and then at 1,000x under oil immersion. The smears were air dried, fixed in methanol for about 1 min, then washed and stained for 45 min in 10% (V/V) Gurrs R66 improved Giemsa in phosphate buffer at pH 7.2.

2.3.2 Administration of N. locustae to Locusta migratoria

A suspension of 1 x 10^5 N. locustae spores was fed directly to each of 100 3rd instar L. migratoria nymphs, by placing 1 µl by micro pipette onto the mouth parts of the nymph, which then ingested it, usually with regurgitated digestive fluid. A group of 50 3rd instar nymphs were reared spore free·(It was expected that some of the infected nymphs would die).

Ten days after infection, 2 nymphs were dissected daily and fat tissues were removed and checked for infection as before.

2.3.3 Administration of N. locustae to O. praemonatus, O. annulipes and C. sylvestrii

Chinese cabbage leaves were immersed in a spore suspension of N. locustae, containing 1 x 10^5 spores/µl and left until the suspension started to dry. They were then

offered to 2nd instar nymphs of the grasshoppers. Third instar nymphs of O. annulipes and C. sylvestrii were offered leaves after immersion in a spore suspension containing 1 x 10^7 spores/µl and 5th instar O. annulipes were offered leaves which had been immersed in a spore suspension containing 1 x 10^9 spores/µl. All the contaminated leaves were left with the grasshoppers for 24 h then the hoppers were offered fresh grass.

Dead grasshoppers were removed and checked for infection by examination of fresh and stained smears of gut and fat tissue as before.

Live hoppers were also checked for infection by taking a drop of blood; after cutting the tarsi of one of the hind legs 8 days after treatment with the spores. The blood was checked at x400 and at x1,000.

Some infected nymphs of O. praemonatus, O. annulipes and C. sylvestrii were dissected to obtain fat tissue, fore-, mid- and hind-gut, Malpighian tubules, air sacs and gonads which were smeared and stained as before.

2.4 Transovarial transmission

2.4.1 Transovarial transmission in Heliothis zea

Pupae of H. zea, infected and non infected with N. heliothidis, were transferred to clean pots and the sexes identified. Emergent adults were placed in pairs, one male (♂) and one female (♀), into 2.5 x 13 in lamp glass containers (page 61). Four groups were set up: uninfected ♂s and ♀s (Group I), infected ♂s and ♀s (Group II),

uninfected ♂s with infected ♀s (Group III) and infected ♂s with uninfected ♀s (Group IV). Each container was provided with a piece of filter paper as an oviposition site and 10% sucrose solution as food.

Eggs were collected on alternate days and the number laid was recorded. The adult mortality was recorded daily. Samples of approximately 100 eggs per ♀ were removed every 2 days to determine fertility.

Two adult ♀s, 48 h after emergence were dissected and parts of the ovarioles were examined for infection in stained smears. Samples of 10 eggs per ♀ were removed, surface sterilized in 0.2% sodium hypochlorite for 5 min and collected on filter paper. The eggs were then rinsed in 10% formalin solution for 40 min, washed and examined in stained smears to determine prevalence of infection.

Some of the 1st generation larvae derived from surface sterilized eggs of infected females were reared and paired in the same manner as their parents. Fecundity and mortality were recorded for this generation, which had been infected transovarially. After death of adults of both generations, the bodies were individually harvested to determine the number of spores present. The spores were extracted from infected dead adult H. zea as in Section 2.2.1 (page 61).

2.4.2 Attempts to demonstrate transovarial transmission in Locusta migratoria

Mature locusts were separated into 2 groups, uninfected ♂s with infected ♀s and infected ♂s with uninfected ♀s.

After allowing fertilization to take place some adults, ♂s and ♀s, were dissected to obtain mature testes and ovaries. Parts of testes and ovaries were examined for parasite in stained smears. The rest of the adults were left to lay eggs in the oviposition tubes. Some tubes were incubated at 30-32°C to obtain the F_1 generation, which were smeared shortly after they had hatched. From other tubes the egg-pods were collected just after laying. Some eggs were separated from the pods and smeared without treatment, while others smeared after surface sterilization by rinsing in 10% formalin for 45 min then washing in tap water. Some of the froth around the eggs in the pod was also smeared.

2.5 Histological studies

2.5.1 Light microscopy

2.5.1.1 Heliothis zea

Ten larvae of 4th instar, infected either with N. heliothidis, N. bombycis or P. operophterae, were dissected in saline to obtain developing gonads of the ♂s. The gonads were dehydrated, embedded, sectioned and stained as in Appendix 2. The testes dissected from adult ♂s 48 h after emergence were similarly prepared.

Female adults, 48 h old, infected either with N. heliothidis, N. bombycis or P. operophterae were dissected in saline to obtain infected ovarioles which were cut into pieces 5 mm long and were prepared for sectioning as in Appendix 2.

Non-embryonated eggs and embryonated eggs at the black head stage were fixed in alcoholic Bouin's solution at 60°C for 10 min, transferred to cold fixative, punctured with a sharp needle and left in the fixative for 1 h. The blastoderms and embryos were dissected out from the chorion, dehydrated, embedded and sectioned as in Appendix 2. Embryonated sections were stained as in Appendix 2 and sections of non-embryonated eggs were stained with Gram alum/Heidenhain's stain as in Appendix 3.

2.5.1.2 Locusta migratoria

Ovaries and testes of infected adults were dissected in saline and prepared for light microscopy studies as in Appendix 2.

2.5.1.3 Oxycatantops annulipes and Catantops sylvestrii

Male and female gonads of 3rd instar nymphs of C. sylvestrii and 5th instar nymphs of O. annulipes were dissected in saline and prepared for sectioning as in Appendix 2.

2.5.2 For electron microscopy studies

Pieces of ovarioles of adult H. zea, infected either with N. heliothidis, N. bombycis or P. operophterae and pieces of ovarioles of L. migratoria infected with N. locustae, were fixed in Karnovsky's fixative for 10 min at room temperature then for 1 h at 4°C post-fixed in osmium tetroxide, dehydrated in acetone and embedded in Spurr's resin (Appendix 4).

Embryonated eggs were immersed in Karnovsky's fixative for 12 h at 4^{o}C before the embryos were dissected out of the chorion and treated as before. A detailed schedule is given in Appendix 4. Sections (90 - 150 nm) were cut with glass knives on a Reichert 318418 ultramicrotome, mounted on uncoated 300 mesh copper grids, post-stained in Reynold's lead citrate and viewed with a Philips EM 300 electron microscope at an acceleration of 60 KV.

3. RESULTS

Glossary of abbreviations used in the illustrations to sections 3.1 - 3.4

Ap: Apical cell

B : Binucleate stage

C : Cuticle

DB: Degenerate Binucleate stage

DS: Degenerate spore

ED: Epidermis

EP: Epithelial Sheath

ER: Endoplasmic Reticulum

ES: Enclosing sheath of the Testicular Cyst

FC: Free cells

FE: Follicular Epithelium

G : Golgi apparatus

GE: Gut Epithelium

GT: Genital Tubule

IFT: Interfollicular Tissue

IT: Intertubular Tissue

M : Mitochondria

N : Nucleus

n : Nucleolus

ND: Nodule

NG: Nerve Ganglia

OES: Outer Epithelial Sheath

OO: Oocyte

P : Polar Tube

PC: Pocket of Parasite

PE: Periplasm

PP: Polaroplast

PS: Peritoneal Sheath

S : Spore

SC: Testicular Cyst

SE: Serosa

SG: Spermatogonia

SM: Spindle Microtubules

SP: Sporont

SPB: Sporoblast

SPD: Spermatid

SR: Sporogonic Stages

ST: Sperm Tubule

t : tubule

TC: Testicular Cyst cells

TP: Tunica Propria

TR: Trophocyte

V : Vitelline membrane

VA: Vacuole

VE: Vesicle

YG: Yolk Granule

Z : Spermatozoa.

Nosema heliothidis

3.1.1 The effect of N. heliothidis on the reproductive capacity of Heliothis zea

The mean numbers of eggs oviposited by uninfected females and females which had been infected at 3 dose levels (1.0×10^6, 3.2×10^6 and 4.4×10^6 spores/larva) then mated with uninfected males or males infected with the same doses are given in Table 1. Oviposition for individual females are given in Appendix 5 (pages 214,215). The mean number of eggs oviposited by uninfected females (Groups I and IV together) was 556. The corresponding figure for infected females (Groups II and III together) was 274. There is a significant difference between the total number of eggs oviposited by uninfected and infected females ($P<.001$) (3 way analysis of variance, F test).

The mean numbers of eggs laid by females whether infected or uninfected, when mated with uninfected males (Groups I and III) and infected males (Groups II and IV), were 532.5 and 248.5 respectively. The difference is again significant ($P<.001$, F. test).

In 3 separate experiments, doses of 1.0×10^6, 3.2×10^6 and 4.4×10^6 spores/larva were used to study the effect of dose on the reproductive capacity. The mean numbers of eggs of infected females (mated either with infected or uninfected males) were 200.5, 284 and 187.5 for the 3 doses respectively (Table 2). The differences between these means were not significant (F test).

Table 1

Effect of *N. heliothidis* on female fecundity of *H. zea* :

mean numbers of eggs laid by female moths

Experiment Number	Both Partners uninfected		One Partner Infected		
	Group I	Dose (Spores/ larva)	Group II	Group III	Group IV
1	691 (n=8)	1.0×10^6	263 (n=6)	138 (n=7)	331 (n=8)
2	785 (n=7)	3.2×10^6	319 (n=9)	249 (n=9)	490 (n=5)
3	765 (n=10)	4.4×10^6	114 (n=7)	261 (n=10)	275 (n=10)
Mean eggs laid	747	-	232	316	365
Mean eggs laid by infected or uninfected females		(Group I & Group IV) 556		(Group II & Group III) 274	
Mean eggs laid by females mated with infected or uninfected males		(Group I & Group III) 532.5		(Group II & IV) 248.5	

n = number of females tested.
Group I = Uninfected males x uninfected females.
Group II = Infected males x infected females
Group III = Uninfected males x infected females
Group IV = Infected males x uninfected females.

Table 2

Effect of doses of *N. heliothidis* on the reproductive capacity of *H. zea*

Dose	Uninfected male x Infected female	Infected male x Infected female	Mean
1.0×10^6	138	363	200.5
3.2×10^6	249	319	284
4.4×10^6	261	114	187.5

3.1.2 Spore load of Nosema heliothidis in Heliothis zea

The final spore load was investigated in 48 adult insects infected as 3rd instars with doses of $1x10^6$ spores/larva by harvesting the spores after the insects had died. The number of spores harvested ranged from $4x10^7$ in 3 insects to $2x10^8$ in 2 insects. The results are represented in Fig.11.

3.1.3 Relation between spore load and fecundity

As the fecundity might be related to the final spore load in the adult insect rather than to the initial spore dose, an attempt was made to determine such a relationship. Taking adult females which had been given doses of $1x10^6$ spores and mated with uninfected males, spores were harvested from 12 individual survivors after they had laid their eggs. No correlation was found between the spore load and reproductive capacity (correlation test) Fig.12. Details of the spore loads and oviposition on individual female moths are given in Appendix 7 (page 220). The spores were also harvested from 23 adult females which had been infected transovarially and mated with uninfected males. The spore load was compared with the total number of eggs laid by these females. Again there was no correlation between spore load and fecundity in the insects infected transovarially (Fig.13). Details of the spore loads and oviposition of individual female moths are given in Appendix 8 (page 221).

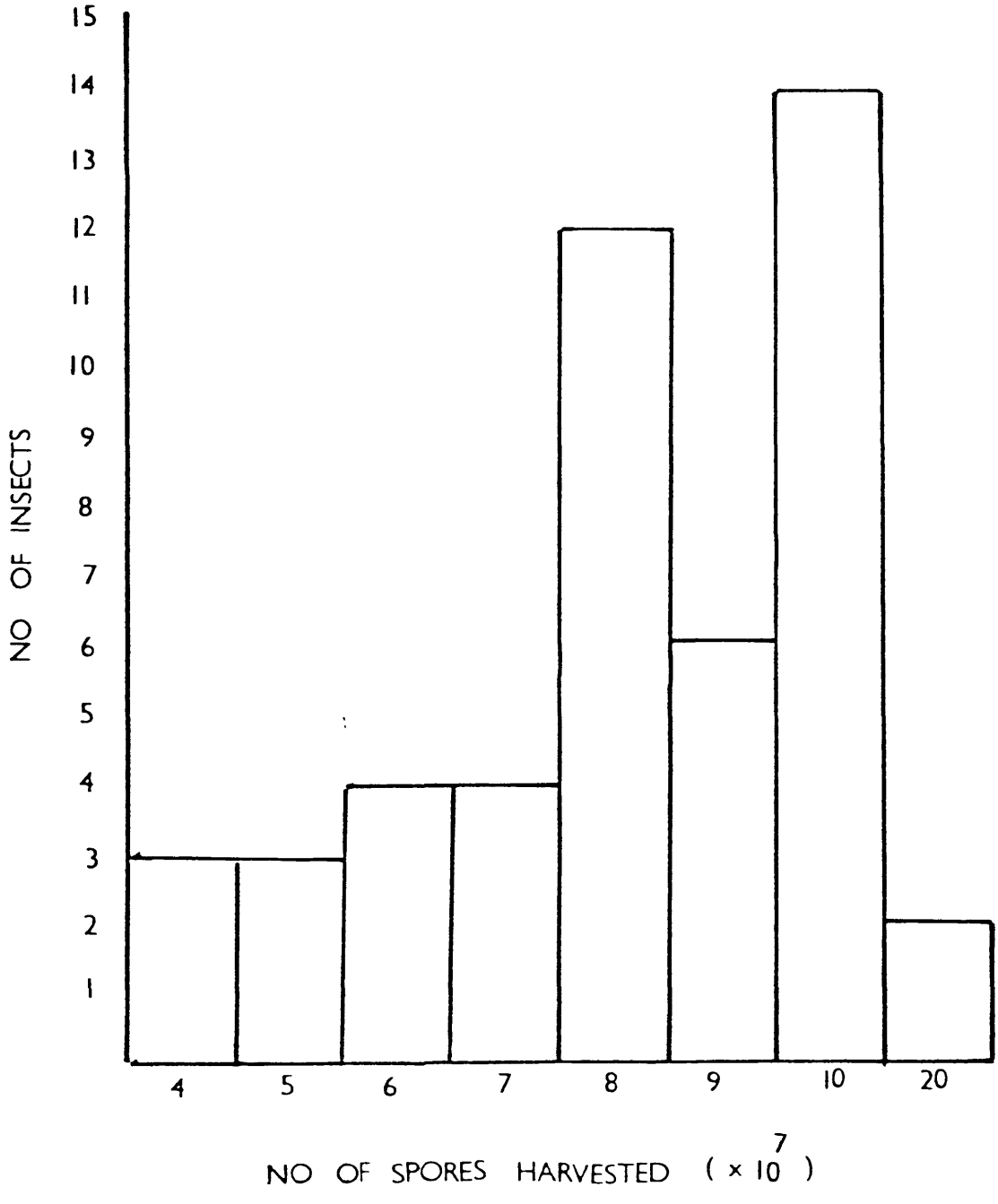


FIG 11

The number of spores harvested from individual H. zea which had been infected with 1 x 10^6 spores at the 3rd instar.

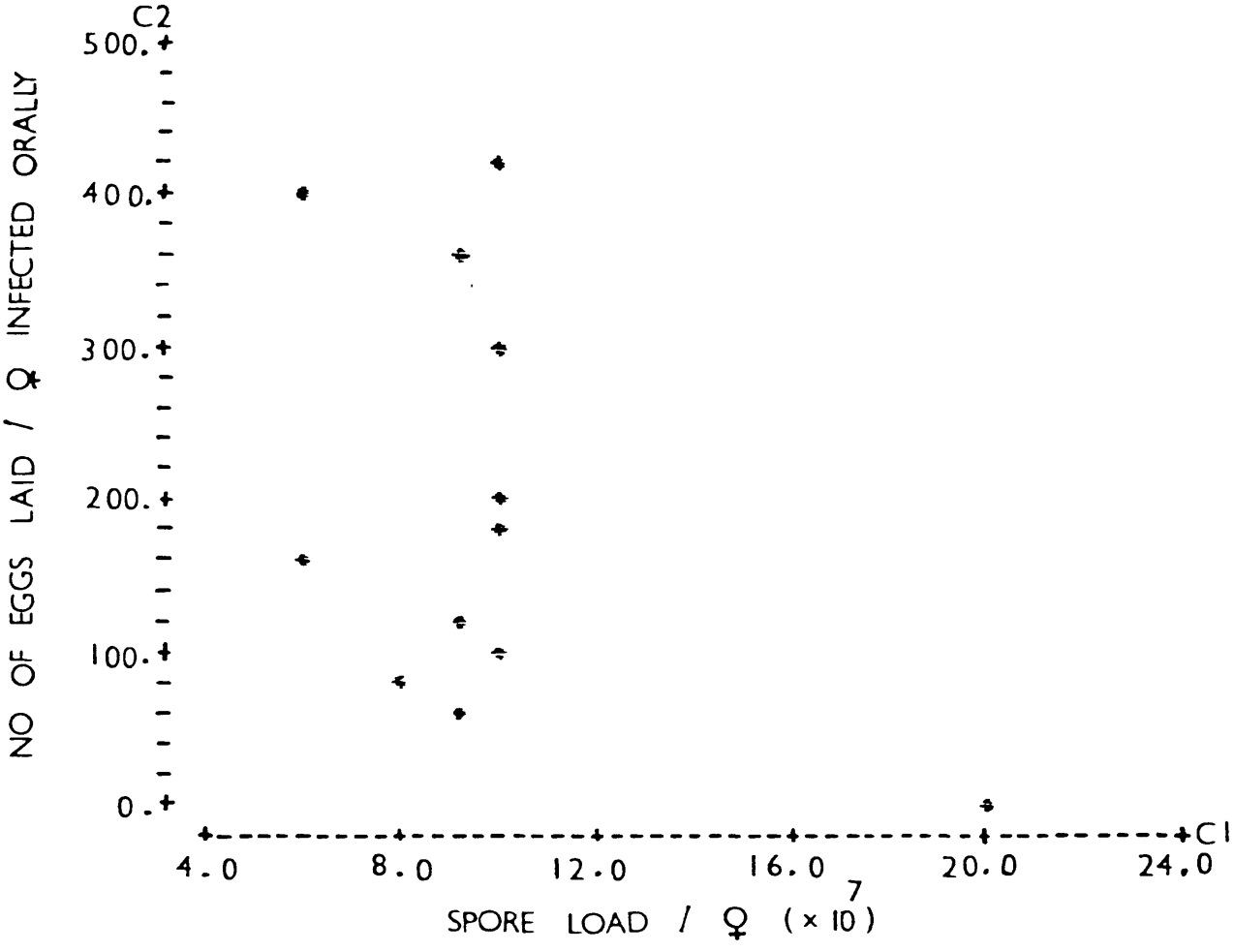


FIG 12

Relation between spore load of N. heliothidis and number of eggs laid by H. zea.

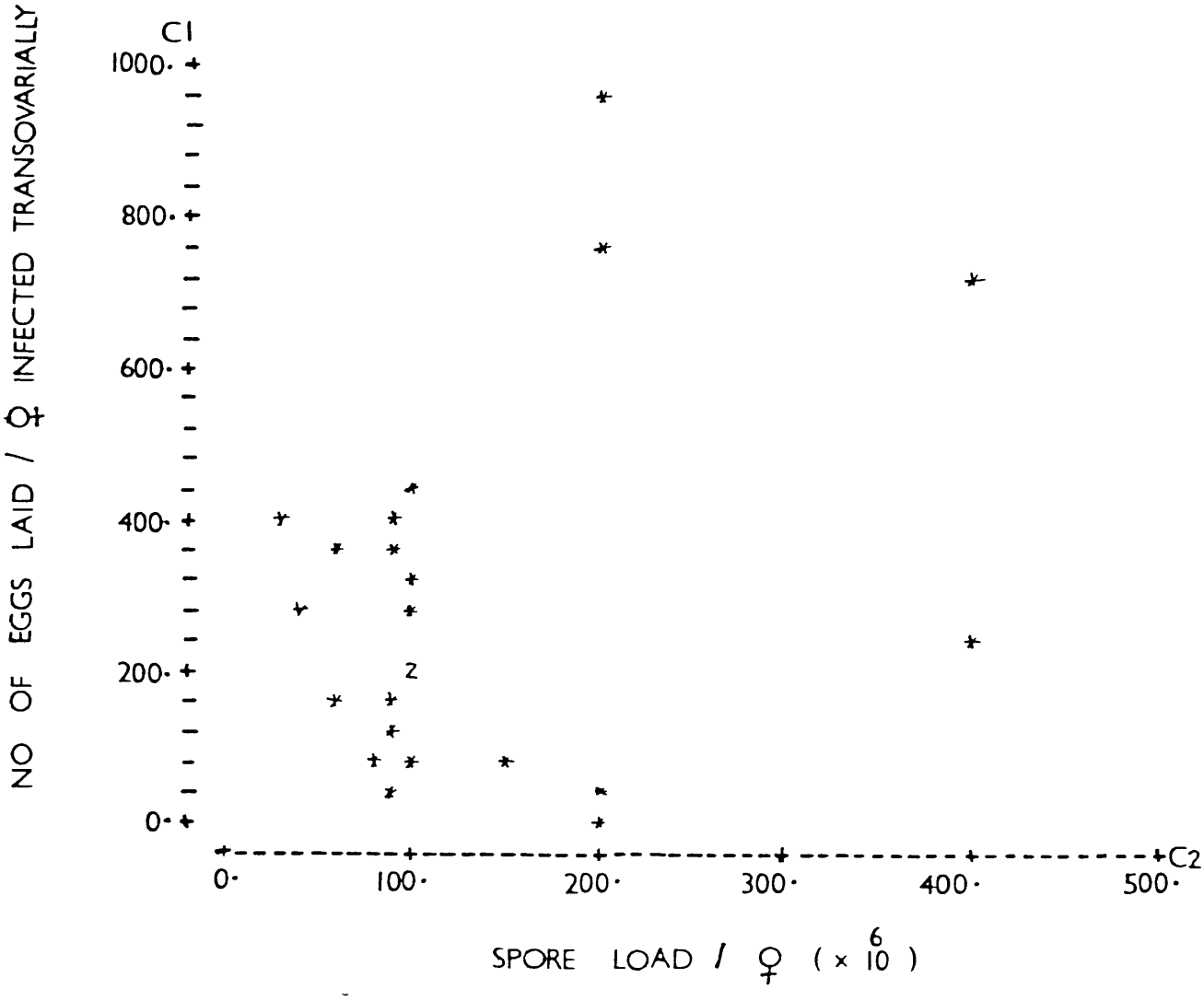


FIG 13

Relation between spore load of N. heliothidis and eggs laid by females of H. zea infected transovarially

3.1.4 Relation between spore load and total number of eggs formed

The number of eggs laid together with the unlaid eggs (eggs which remained in the ovarioles after death) was recorded for a number of females which had been infected transovarially. These figures were compared with corresponding spore load and again there was no correlation either with the total number of eggs formed (Fig.14 and Table 3) or with the number of unlaid eggs (Fig.15) (Correlation test).

Table 3

Comparison between the total number of eggs formed and the spore load

Number of eggs laid	Number of unlaid eggs	Total number of eggs formed	Total number of spore load
140	160	300	1.8×10^8
530	185	715	1.9×10^8
285	130	415	2.3×10^8
140	130	270	2.6×10^8
95	80	175	2.6×10^8
120	360	480	2.7×10^8
1385	55	1440	3×10^8
900	35	935	3.6×10^8
905	85	990	3.7×10^8
49	30	520	3.9×10^8
50	310	360	4×10^8
640	205	845	4.2×10^8

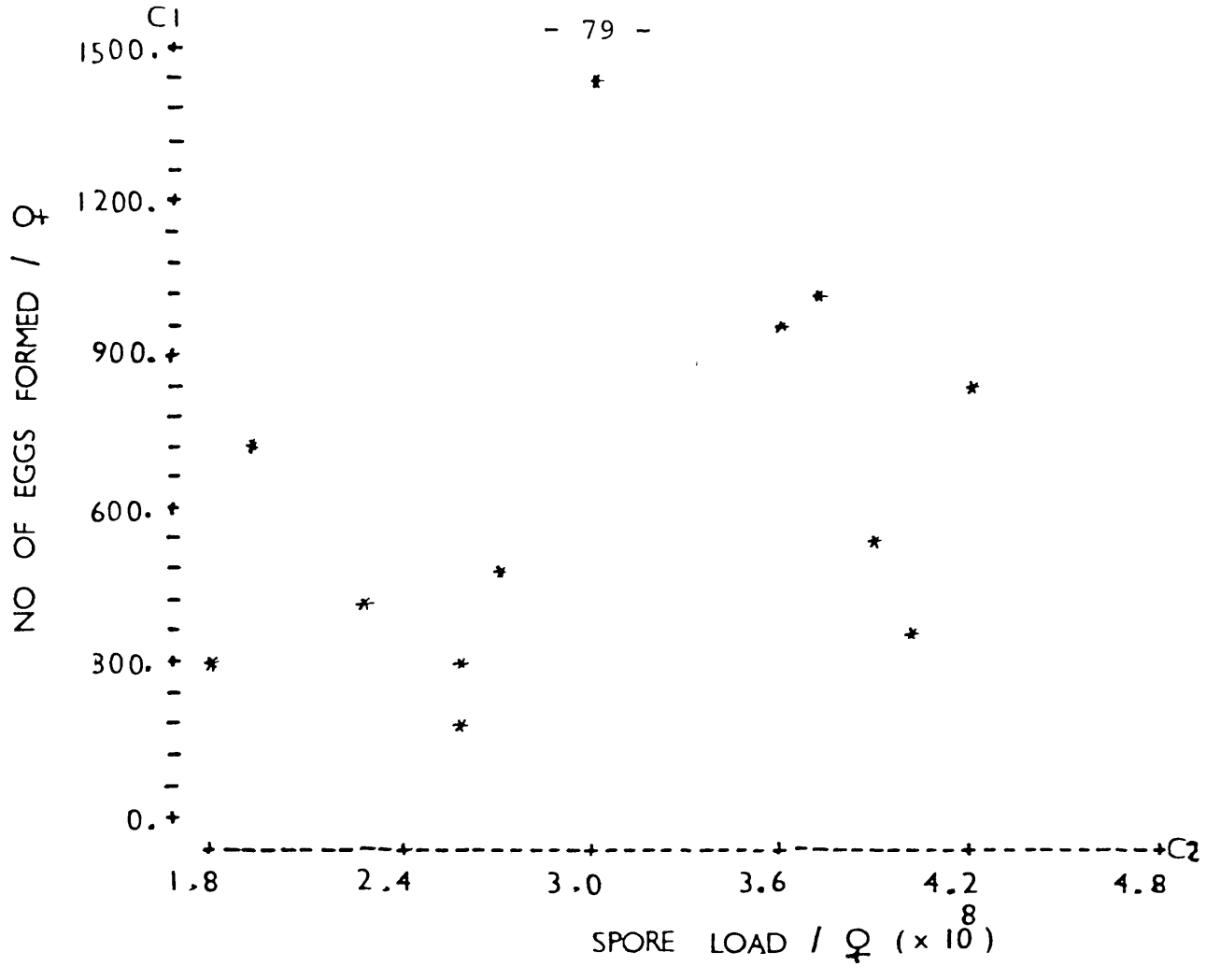


FIG 14

Releation between eggs formed in female H. zea and spore load of N. heliothidis

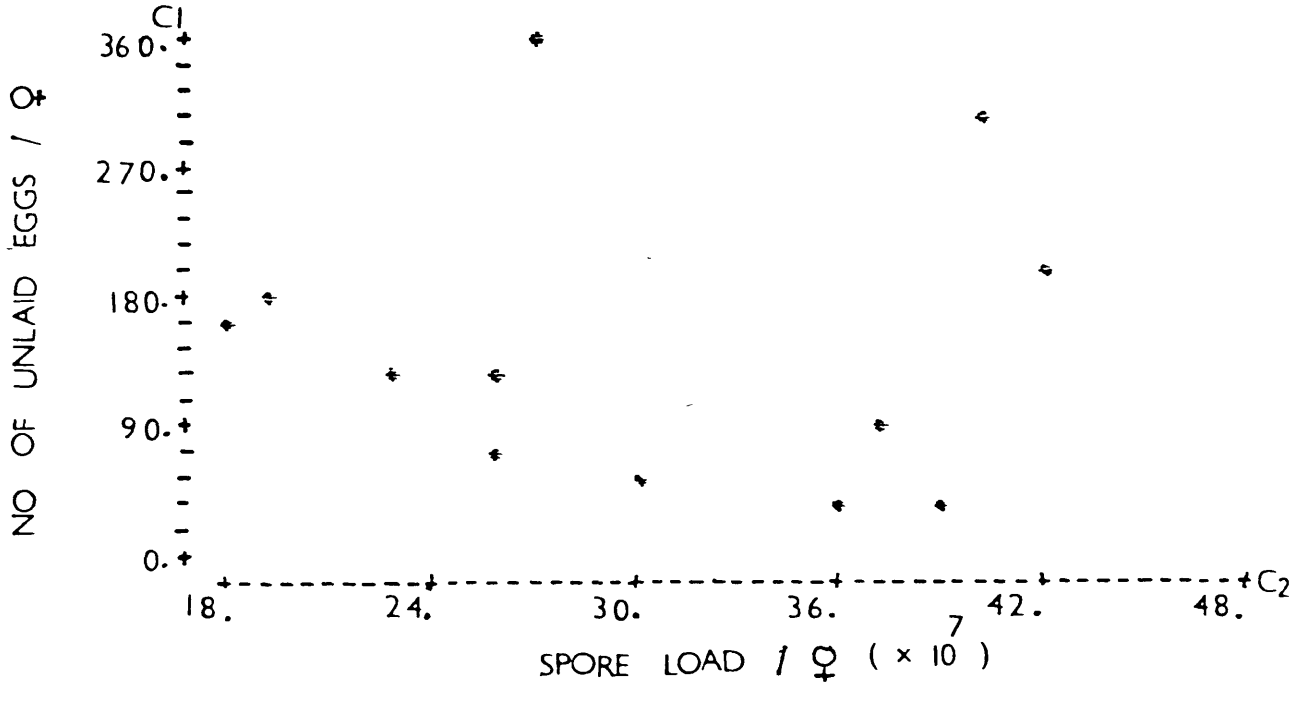


FIG 15

Relation between spore load of N. heliothidis and unlaid eggs of females H. zea.

3.1.5 Comparison of the spore load in insects infected orally and transovarially

To compare levels of infection between mothers and their progeny, spores were harvested from some females which had been infected orally and from some of their progeny infected transovarially. The results are represented in Fig.16. Details from individual insects are given in Appendix 9 (page 222). It is obvious that the spore load of the progeny, in general, is greater than that of their mothers and the difference is significant (correlation test), but there is no correlation between the individual spore load in any one mother and spore load in progeny of that mother.

3.1.6 Relation between spore load of males, females and percentage of egg hatch in adult H. zea infected transovarially

The numbers of spores harvested from males and from the females with which they had mated were compared with the percentages of eggs which hatched. There was no correlation between the spore loads of females and the percentage of their eggs hatching (Fig.17) but there was a strong correlation between the spore loads of males and the percentages of eggs hatching from that mating ($P<.025$, correlation test) i.e. the higher the spore load in the males, the lower, was the percentage of eggs hatching from the mating (Fig.18). Details of spore loads and percentage of egg hatch from individual male and female H. zea are given in Appendix 10 (page 223).

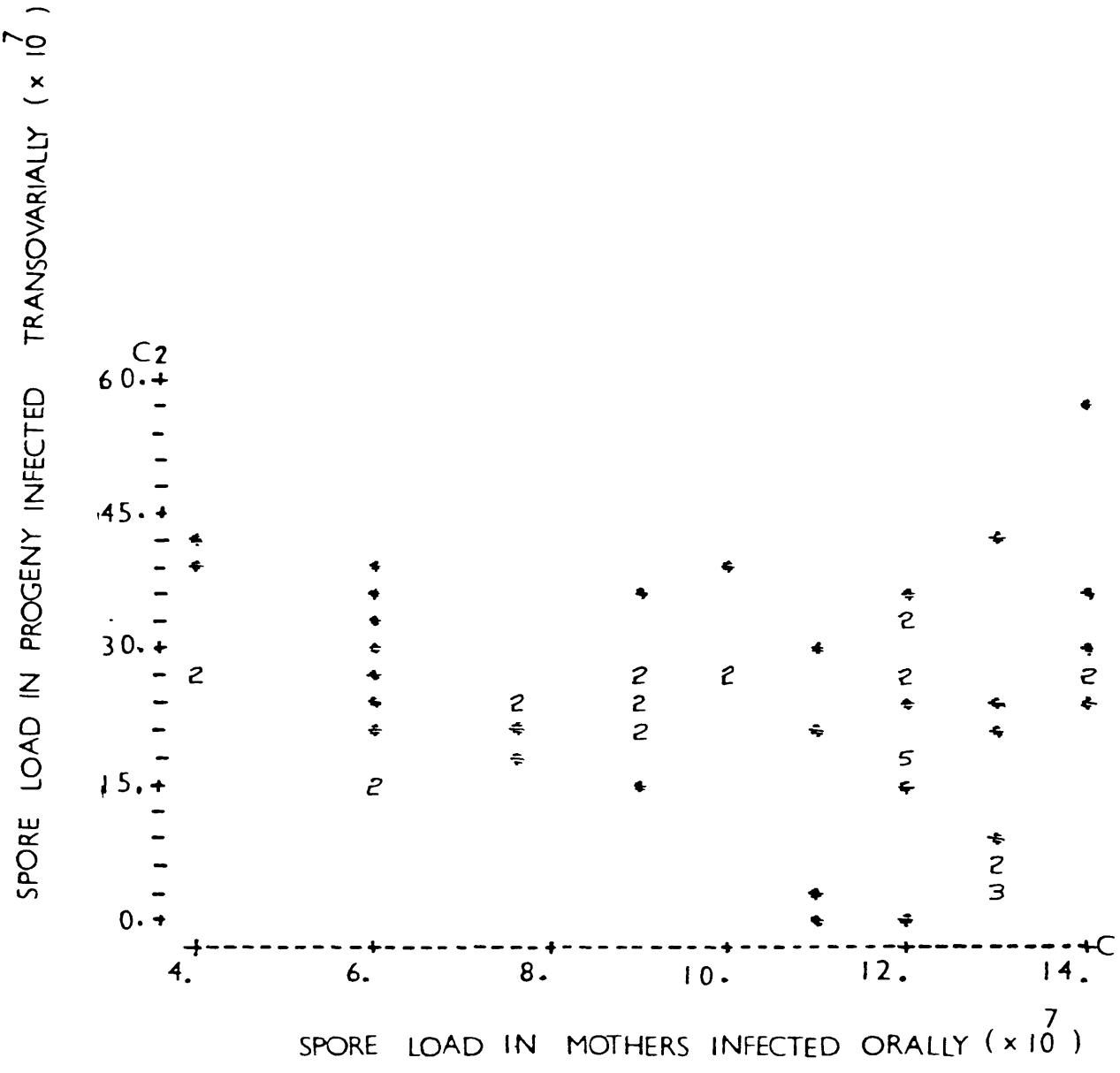


FIG 16

Relation of spore load of N. heliothidis in mothers and their progeny of H. zea.

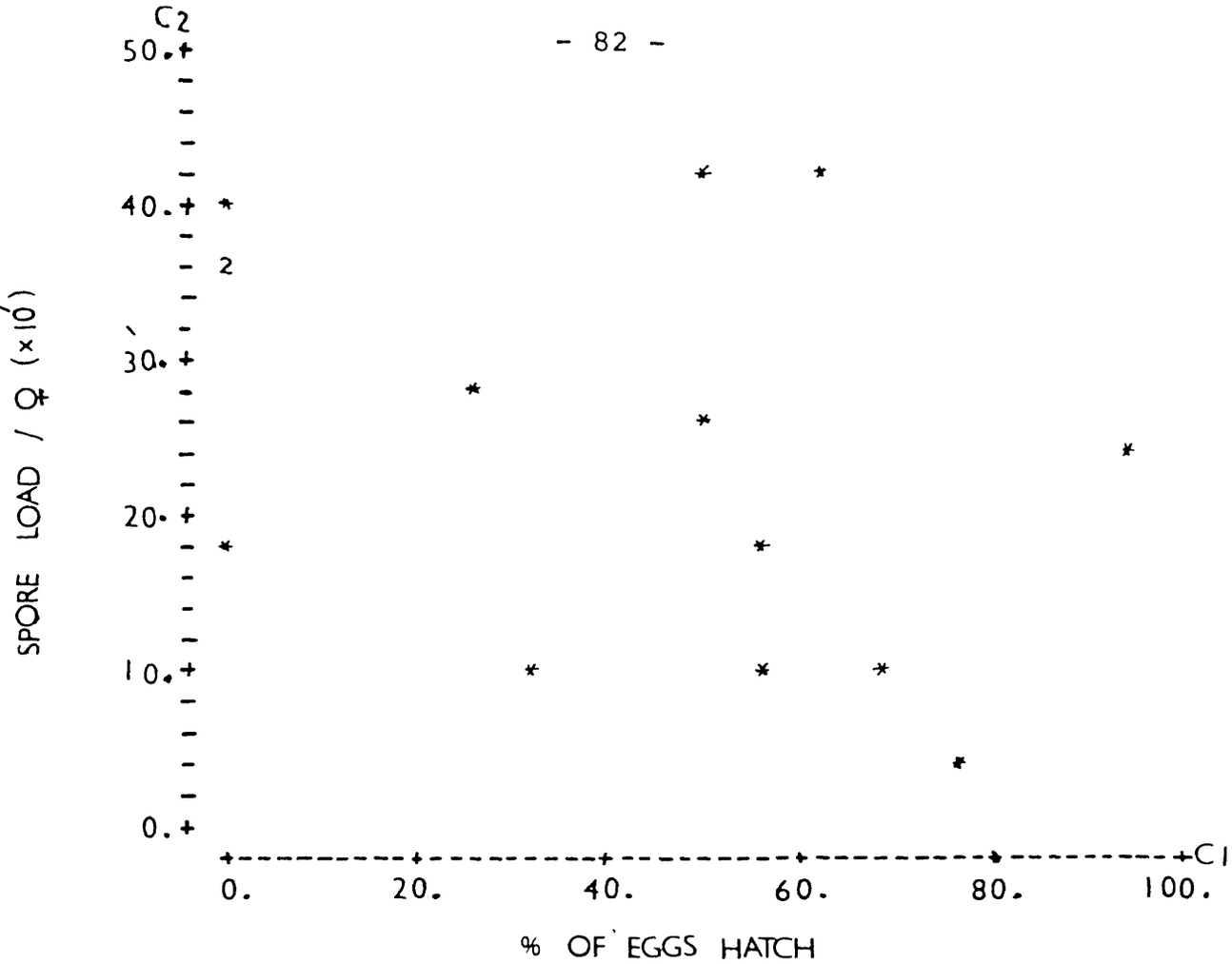


FIG 17

Relation between spore load/female and percentage of egg hatch

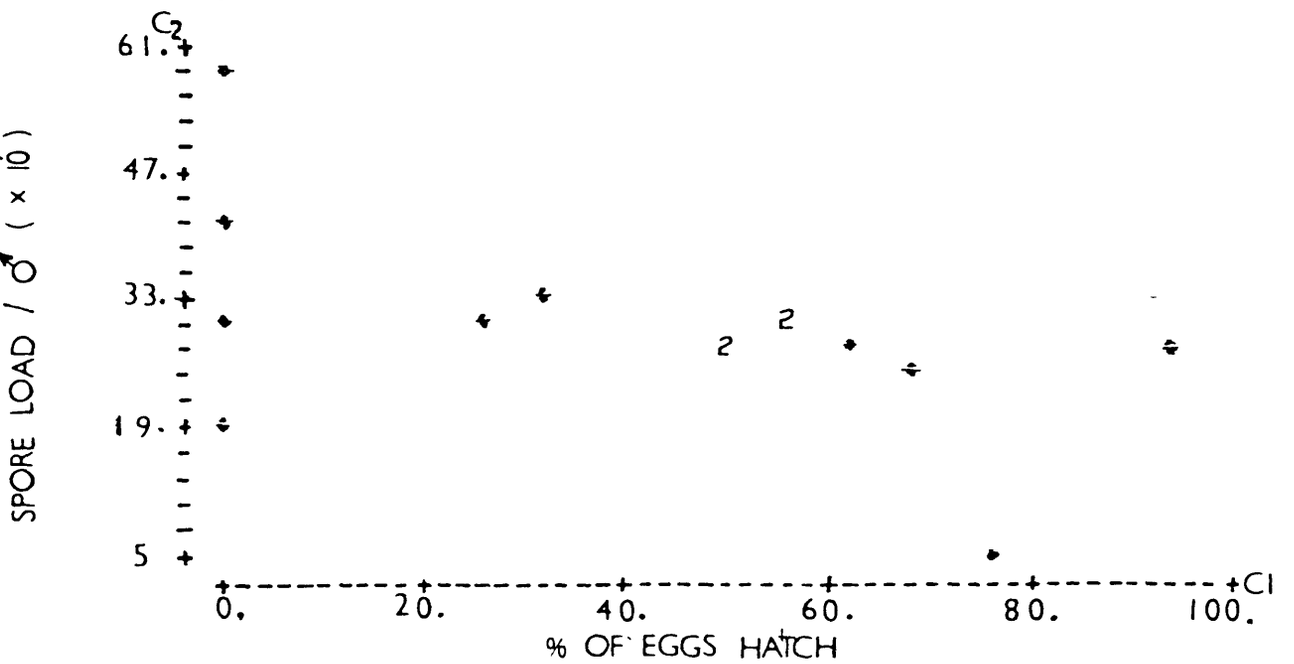


FIG 18

Relation between spore load/male and percentage of egg hatch.

3.1.7 Effect of N. heliothidis on the life cycle of H. zea

Doses of 1.0 x 10^6 and 3.2 x 10^6 spores/larva caused a slight increase in larval longevity (from egg hatch to pupa formation). The elongation was 1 day for insects treated with dose 1 x 10^6 spores/larva and 2 days for insects treated with dose 3.2 x 10^6 spores/larva. Insects treated transovarially had only 1 day elongation in larval longevity. Adult longevity was reduced by 3.3% and 6.4% for insects treated orally, with doses 1 x 10^6 and 3.2 x 10^6 spores/larva respectively, and reduced by 4.0% for insects infected transovarially (Tables 4 and 5). Details from individual insects are given in Appendix 6 (pages 216-219).

Table 4

Effect of N. heliothidis (doses 1x10^6 spores/larva) on life cycle of H. zea

Treatment	Average of life cycle	larval long-evity	larval longevity as % of total life cycle	Adult long-evity	Adult longevity as % of total life cycle	Reduction of adult longevity due to infection %
Un-infected	41.105 ±1.91	18.000 ±1.19	43.791 ±3.46	9.316 ±1.63	22.548 ±2.99	-
Infected with dose 1x10^6 spores/larva	40.444 ±2.34	19.458 ±1.03	48.112 ±2.53	7.889 ±2.44	19.236 ±5.18	3.312

Table 5

Effect of N. heliothidis on life cycle of H. zea infected orally (dose 3.2 x 10^6) and transovarially

Treatment	Average of life cycle	larval long-evity	larval longevity as % of total life cycle	Adult long-evity	Adult longevity as % of total life cycle	Reduction of adult longevity due to infection %
un-infected	44.800 ±2.94	18.452 ±1.61	41.179 ±2.631	12.067 ±3.23	26.969 ±5.70	-
Infected with dose $3.2x10^6$ spore/ larva	44.267 ±3.15	20.243 ±1.52	45.751 ±3.06	9.300 ±2.95	20.612 ±6.05	6.357
Un-infected progeny	46.419 ±5.14	16.576 ±1.37	35.979 ±4.22	15.161 ±4.45	32.228 ±7.42	-
Infected progeny	45.771 ±3.73	17.257 ±1.22	37.989 ±4.53	13.143 ±3.91	28.202 ±7.13	4.026

3.1.2 Histological studies of the reproductive systems of Heliothis zea infected with Nosema heliothidis

The testis of 5th instar larvae (Fig.19) consists of 4 sperm tubules separated from each other by intertubular tissue. This is seen in longitudinal section in Fig. 19. Each sperm tubule consists of a great number of testicular cysts and one apical cell. Each testicular cyst has a number of spermatogonia. The entire organ is ensheathed by the peritoneal sheath. In adult male moths the 2 testes are bounded together in a single capsule and consist of a large number of incompletely separated tsticular tubules

(Fig.20). Each testicular tubule consists of cysts containing spermatogonia, spermatocytes, spermatids and spermatozoa. The cysts are bounded by testicular cyst cells.

In male larvae which were infected orally at 3rd instar with 4.4×10^6 spores/larva, N. heliothidis started to invade the peritoneal sheath of the testes during the 4th instar. Invasion of the sperm tubules began in the early 5th instar. The parasites were scattered throughout the peritoneal sheath (Fig.21) and the intertubular tissue but appeared in groups in the testicular cysts (Fig.22).

By the time similarly treated H. zea had become adult, N. heliothidis had extensively invaded the peritoneal sheath, intertubular tissue and testicular cyst cells of the testes (Figs.23 and 24). In heavily infected testes, spores and vegetative stages were seen in all testicular tissues. Peritoneal sheath and intertubular tissues were uniformally infected. The testicular cyst cells, which limit the developing spermatogonia, spermatocyte, spermatids and spermatozoa, were heavily invaded and were sometimes totally destroyed.

The ovary of adult female of H. zea is of the polytrophic type. The vitellarium region has trophocytes and the oocytes lined up in a single row to form follicles. Each follicle consists of 6 trophocytes and 1 oocyte, surrounded by follicular epithelium.

Ovaries of 5th instar larvae of H. zea were not examined. All the tissues of the ovary of adult moths,

except the follicular epithelium, were invaded by N. heliothidis. A few spores were seen in the germarium and some were scattered in the interfollicular tissue, trophocytes (Fig.25) and oocytes in the vitellarium region. Stages could be recognized, by light microscopy, as spores and binucleate forms which could have been meronts or sporonts but it was not possible to distinguish between them. Binucleate stages were seen in the oocyte concentrated around the periphery (Fig.26) and in clumps towards the centre (Fig.27), rather than evenly distributed throughout the oocyte cytoplasm. Binucleate stages were obscured by the cytoplasmic contents of the trophocytes in immature follicles. In premature follicles, in which the trophocytes had been nearly absorbed by the oocytes, binucleate stages were quite easily distinguished among the remains of the trophocytes (Fig.28). The trophocytes were moderately invaded.

Electron microscopy revealed greater detail of parasite structure and their relationship with the host cells. Sporonts were distinguished from meronts by the thickness of the limiting membrane. Meronts being limited by a thin plasma membrane, while sporonts had electron-dense material deposited on their limiting membrane. Meronts were frequently seen in immature oocytes (Fig.29) but were seen to a lesser extent in interfollicular tissue and trophocytes. Early sporonts were seen in oocytes, trophocytes and interfollicular tissue (Figs.30-32). Late sporonts

were seen in mature oocytes (Fig.33). Few sporoblasts were found in trophocytes (Fig.34). In young oocytes sporoblasts were not seen but they were seen in mature oocytes (Fig.35). Degenerate spores were seen frequently in interfollicular tissue (Fig.36) and a few in oocytes but non in trophocytes. Well developed spores were not present in any of the ovarian tissues - oocytes, trophocytes, or interfollicular tissue. The epithelial sheath was also invaded by the infective stages of the parasite (Fig.37) and, in fact, the follicular epithelium was the only tissue of the ovariole which was not invaded by the parasite.

In smears of recently laid non-embryonated eggs, many binucleate stages of N. heliothidis could be seen but their distribution could not be determined (Figs.38,39). However, in histological sections, the binucleate stages could be seen arranged isolated or in small pockets, scattered throughout the cytoplasm (Figs.40,41). No spores were seen either in smears or sections of non-embryonated eggs. In embryonated eggs binucleate stages were found among the remnants of the yolk beneath the serosa (Fig.42) and at black-head stage a few spores were seen in connective tissue, muscles and nervous tissue (Fig.43). A number of spores were also seen among the yolk granules which filled the fore- and midgut of the larva (Fig.44).

Examination of 10 eggs, from each of 60 adult female H. zea, showed that there was 100% transmission to the progeny in 51 insects, 90% in 6 insects, 70% in 2 insects and 0% in

l insect. Non-embryonated eggs of healthy females paired with infected males examined in smears were devoid of infection.

Electron microscopic studies revealed that the spores which were present in the embryonated eggs were well developed and invaded all the embryonic tissues even in epidermal cells (Fig.45). They were also found among yolk granules which filled the gut of the embryo before hatching from the egg (Fig.46). Meronts were not seen in the embryonic tissues but stages with a thick surface coat, identified as sporogonic stages, were present alongside the spores. Degenerate vegetative stages were found among the remnants of the yolk outside the embryo within the egg shell (Fig.47).

3.1.3 The ultrastructure of Nosema heliothidis

Meronts

Meronts were rounded or slightly elongate cells, bounded by a simple plasma membrane. Nuclei were in diplokaryon form, closely apposed to one another but each was surrounded by its own double envelope. There were numerous nuclear pores. The envelopes appeared dense in the contact area due to the apposition of the four membranes (Fig.48). In some cases the cytoplasm was conspicuously less dense than nucleoplasm (Fig.49) and vice versa in others. There were ribosomes and flattened cysternae of rough endoplasmic reticulum (Fig.48). Meronts divided by simple binary fission (Fig.50). Nuclei were not seen in division. The

parasite lay in direct contact with the oocyte, trophocyte and interfollicular cytoplasm, which contained mitochondria. Some meronts had numerous mitochondria around their surfaces (Fig.51). Tubules were present in direct contact with the host cytoplasm lying in parallel bundles close to the parasite surface. They measured about 26 nm in diameter and at least 0.5 µm in length. Some material of moderate electron density filled the lumen (Fig.49).

Sporonts

The sporont envelope was thickened by deposition of electron dense material on the outside of the plasma membrane. The electron dense material was initially laid down at the poles then spread to cover the entire sporont (Fig.52). Mature sporonts were elongated. The cytoplasm of the host surrounding the sporonts was often completely disrupted and tubules of 20 nm diameter were found scattered around the sporonts in the space created (Fig.53). Division of the sporont into sporoblasts was not seen.

Sporoblasts

The surface coat of the sporoblast was thicker than that of the sporont. No evidence of development of the polar filament and polaroplast complex was seen in the young sporoblast (Fig.54). Tubules of 20 nm diameter were seen in spaces created by degeneration of the host cell cytoplasm around the sporoblasts, as with the sporonts. The internal structures of mature sporoblasts were not well preserved (Fig.55).

Spores

Spores were not well preserved, as it is difficult for fixatives to penetrate the spore wall but sufficient detail was seen to demonstrate that fully mature spores were present.

The spore wall was composed of an electron-dense exospore 20 nm thick and an electron-transparent endospore 130 nm thick. The nuclei were not well preserved. The polar tube consisted of a straight basal part and about 10 coils (Figs.56,57). At least 3 concentric layers could be seen in the tube section around a central core. The angle of tilt was not measured because no complete longitudinal sections in the right plain were obtained. The polaroplast consisted of two parts, an anterior part composed of compact membranes and a posterior part made up of loosely stacked flattened sacs (Fig.56).

3.2 Nosema bombycis

3.2.1 Histological studies of the reproductive system of Heliothis zea infected with Nosema bombycis.

When 2nd instar larvae of H. zea were infected orally with 2×10^5 spores of Nosema bombycis, the testes were invaded in the late 5th instar. The peritoneal sheath and intertubular tissue of the testes were heavily infected and the infection was distributed throughout these tissues. Spores and vegetative stages of N. bombycis were seen in clusters infecting the enclosing sheath of the testicular cysts which are a constituent of the sperm tubules (Fig.58,

59). A few of the spermatogonia which develop in the testicular cysts were invaded (Fig.58). Ovaries were not histologically examined.

Larvae which were infected orally at 2nd instar either with 4×10^5, 2×10^4. 6×10^3 spores/larva died as pupae. Fifth instar larvae which were dosed either with 6×10^3, 4.8×10^2 or 40 spores/larva became adult but failed to emerge from the puparium. These adults were dissected out of the puparium and the genital systems were histologically examined.

Spores and binucleate stages of N. bombycis invaded the peritoneal sheath and intertubular tissue of the testes of adult males. The testicular cyst cells which limit the spermatids and spermatozoa were infected with spores and binucleate stages of the parasite (Figs.60,61).

Adult females which had been infected during the 5th instar with 6×10^3 spores/larva had heavily infected ovaries. In partly developed follicles (Fig.62) the trophocytes were heavily infected with spores which also filled the cytoplasm of the oocyte. Even the follicular epithelium was invaded and instead of being single layered as in the uninfected follicles, it became multilayered. In late follicles the follicular epithelium was completely destroyed and mature ova were not enclosed by the vitelline membrane. Ovaries of adult females which were infected in the 5th instar with 4.8×10^2 were less heavily infected. A few spores were seen in trophocytes and oocytes of young

follicles but the follicular epithelium was not infected. Mature oocytes were lightly infected and the infection was concentrated in the centre of the oocyte (Fig.63). Vegetative stages were not visible in sections of either heavily or lightly infected follicles but smears contained a number of binucleate stages.

Electron microscopy revealed the presence of meronts, sporonts, sporoblasts and spores throughout the ovarial tissues. All the developmental stages of the parasite were seen in the cytoplasm of the oocytes (Fig.64) and trophocytes (Fig.65) of immature follicles. In mature follicles the sporoblasts and spores were the main stages observed. The spores were well developed (Fig.66) but some degenerate spores were seen in interfollicular tissue (Fig.67). No degenerate spores were seen in the trophocytes or oocytes. The follicular epithelium of immature and mature follicles which was comprised of a single layer of cells as in uninfected follicles was lightly infected (Fig.68). The epithelial sheath was also infected with different stages of the parasite (Fig.69).

3.2.2 The ultrastructure of N. bombycis in ovarian tissue of Heliothis zea

Meronts

Meronts were oval and lay in direct contact with host cell cytoplasm. They were limited by a thin plasma membrane and contained abundant ribosomes and 2 nuclei in diplokaryon arrangement. The nuclei had electron dense

material suggesting chromosomes but division spindles were not seen (Fig.70). Division was by binary fission (Fig.71).

Sporonts

Immature, binucleate sporonts had somewhat pointed ends. An amorphous electron-dense coat was deposited on the external surface of the plasma membrane starting at the poles and progressing towards the middle (Fig.72). An extensive network of endoplasmic reticulum also distinguished these stages from the meronts. Condensed chromatin indicated that the diplokaryon was entering division. In mature sporonts the surface coat was continuous. There were two diplokarya and division was by binary fission into sporoblasts (Fig.73).

Sporoblasts

Young sporoblasts had a similar organization to sporonts but were regularly ovoid and each nucleus of the diplokaryon had a prominent nucleolus (Fig.74). Stages were seen with transverse and longitudinal sections of the polar tube and a network which has been interpreted in other microsporidia as a Golgi apparatus (Sprague and Vernick, 1969; Milner, 1972; Canning and Nicholas, 1974; Colley, Joe, Zaman and Canning, 1975; Percy, Wilson and Burke, 1982) responsible for secretion and organization of the polar tube (Figs.74-76). The Golgi network gave way to a prominent posterior vacuole in late sporoblast (Fig.77). The polar tube consisted of an electron dense sheath and an electron lucent layer surrounding a central core (Fig.77).

Spores

Spores were not well preserved but some showed the electron-dense exospore 30 nm thick, electron transparent endospore 95 nm thick, reduced to 20 nm at the anterior end (Fig.78) and the polar tube composed of 13 or 14 coils in a single rank (Fig.79). Complete longitudinal sections of the spores were not obtained so the angle of tilt could not be determined.

3.3 Pleistophora operophterae

3.3.1 Replication of P. operophterae in Heliothis zea

H. zea were infected during the 2nd instar with doses 2 x 10^4, 2 x 10^5 and 2 x 10^6 spores/larva, 30 larvae for each dose and the mortality is shown in Table 6. The final number of spores present was estimated for each of the 33 adults, all of which had died without emerging from the pupae. These are shown in Fig.80 in relation to infective dose. There was a strong correlation between infective dose and final spores number, in that the lower doses gave consistently lower spore loads than did the higher doses.

Table 6

The survival rate of pupae of H. zea infected with P. operophterae

No of tested larvae	Infected dose	No of dead larvae	No of adults (all died without emerging)	% of adults (all of which died without emerging)
30	2 x 10^4	17	13	45
30	2 x 10^5	17	13	45
30	2 x 10^6	21	9	30
90	-	0	0	100 healthy adult

3.3.2 Invasion of the genital system of H. zea by P. operophterae

Infection of gonads of male larvae, which had been treated during the 2nd larval stage with 2 x 10^5 spores/larva, was first seen in 5th instar larvae. Abundant spores were observed in the peritoneal sheath and intertubular tissue (Fig.81) and the sperm tubules were lightly infected. The infection was seen in the enclosing sheath of the testicular cysts but spermatogonia were not infected.

The peritoneal sheath (Fig.82) and intertubular tissue were more heavily infected in the adult testes and there was invasion of the testicular cyst cells which surrounded the spermatogonia, spermtocytes, spermatids and spermatozoa (Fig.83).

The genital system of female larvae was not examined. Smears of ovarian follicles of adult females which had failed to emerge from pupae, contained free spores as well as a few sporophorous vesicles and vegetative stages. It was seen that the oocytes and trophocytes contained developmental stages and spores in vesicles (Figs.84,85). These stages were identified as meronts, sporogonic stages and spores by electron microscopy and this technique also revealed the same stages in the interfolliclar tissue and epithelial sheath as well as in oocytes and trophocytes (Figs.86-90).

3.3.3 Ultrastructure of *P. operophterae*

Meronts

Meronts were surrounded by a thin plasma membrane and lay within a closely fitting second membrane, which formed the boundary of a vacuole (Fig.91). The origin of the external membrane is not known. Nuclei of meronts were isolated and frequently contained a prominent nucleolus. Nuclear division was not seen but during cytoplasmic division of a meront with two nuclear profiles (Fig.92), the external membrane was seen to divide also and the resulting meronts were individually surrounded by their own vacuolar membranes.

Sporogony was initiated by the laying down an electron dense layer outside the plasma membrane. When the vacuolar membrane was still close-fitting this was difficult to observe (Figs.94,96) but when the cytoplasm was retracted within the vacuole the surface coat was clear (Fig.93). In places the sporont surface remained connected with the vacuolar membrane by fine cytoplasmic strands (Fig.93). Nuclear replication and cytoplasmic fission occurred within vacuole giving several sporoblasts (Figs.95,97,98).

Ultrastructural details of the sporoblasts were not well preserved but it could be seen that each had a single nucleus with a nucleolus (Fig.97). Vacuoles containing the sporoblasts were partly filled with a finely granular matrix in which prominent granules were scattered (Figs.97,98). Development within the vacuole was not synchronous and both

sporoblasts and spores could be present in the same vacuole (Fig.97). The internal organization of the spores was not well preserved.

3.4 Nosema locustae

3.4.1 Invasion of the genital system of Locusta migratoria by N. locustae

The testes of adult Locusta migratoria consist of great numbers of genital tubules, completely separated from each other by the intertubular tissue. Each tubule consists of successive zones of spermatogonia, spermatocytes, spermatids and spermatozoa surrounded by the testicular cyst cells.

Only the intertubular connective tissue of the testes of adults was found to be infected with N. locustae(Figs.99, 100). The testicular cyst cells were completely free of infection as was the epithelial sheath of the sperm tubules.

The panoistic ovarioles of Locusta migratoria consisted of fully developed primary oocytes in the vitellarium region with no trophocytes. The ovariole is enclosed in a tunica propria and outer epithelial sheath (Fig.101).

In heavily infected females the 3 main regions of each ovariole (terminal filament, germarium and vitellarium) were completely free of infection except in the outer epithelial sheath. The parasite formed nodules in the epithelial sheath (Figs.102,103).

Electron microscopic studies confirmed the absence of infection in immature and mature oocytes. The studies illustrated that the nodules were comprised of sporogonic stages and spores of N. locustae (Fig.104).

Eggs laid by infected females mated with uninfected males were examined in smears and were found free of infection as were smears of eggs laid by uninfected females mated with infected males. The froth which surrounded the egg pods laid by females, whether infected or uninfected and mated with infected males was also devoid of infection. First instar nymphs hatching from these eggs when smeared shortly after hatching were free of infection.

3.4.2. Effect of N. locustae on Oxycatantops praemonatus

63 2nd instar nymphs of O. praemonatus were divided into 3 replicates each containing 21 nymphs and fed on Chinese cabbage leaves immersed in a suspension containing 1 x 10^5 spores/µl and allowed to dry. 38.1% of nymphs died 6 days after infection, 47.6% died between days 6-13 after infection and 14.3% died between days 13-19 after infection (Table 7).

Table 7

Rate of mortality of O. praemonatus infected with N. locustae

No.of days after infection	No.of dead nymphs in the 1st replicate	No.of dead nymphs in the 2nd replicate	No.of dead nymphs in the 3rd replicate	Total no. of dead nymphs	% of dead nymphs
0- 6	7	7	10	24	38.1
6-13	10	12	8	30	47.6
13-19	4	2	3	9	14.3
Control	0	0	0	0	0

Fore- mid- and hindgut, Malpighian tubules, air sacs and haemolymph were moderately invaded by N. locustae. Fat tissue was heavily infected. Reproductive tissues were not examined because the insects died before reaching last nymphal stage.

3.4.3 Effect of N. locustae on Oxycatantops annulipes

159 2nd instar nymphs of O. annulipes had been divided into 3 replicates each containing 53 nymphs and they were fed on Chinese cabbage leaves which had been immersed in a suspension of 1 x 10^5 spores/µl and allowed to dry. The nymphs were checked for infection by examining a drop of haemolymph. The uninfected hoppers were separated from the infected ones. 16% of all the treated hoppers died after 32 days of infection. The rest of the hoppers were reinfected as 3rd instars by feeding on leaves which had been immersed in a suspension containing 1 x 10^7 spores/µl and dried. They were checked for infection after a further 8 days. 32% of the treated hoppers died 18 days after the 2nd infection. The rest of the hoppers were reinfected as 5th instars as before using a suspension containing 1 x 10^9 spores/µl. 19.6% of the nymphs were dead 16 days after the 3rd infection. The surviving 80.4% of nymphs were found free of infection when examined as adults. The results are given in Table 8.

Meronts and spores were seen in haemolymph 8 days after infection in nymphs which had been infected as 2nd or 3rd instars. When nymphs had been infected as 5th instars, the

infection was first observed in the haemolymph at 11 days: Malpighian tubules, and tracheae in the thorax were lightly infected, fore-, mid- and hindgut and haemolymph moderately infected, fat tissue was heavily infected but ovaries and testes were found to be uninfected when examined in sections.

3.4.4 Effect of N. locustae on Catantops sylvestrii

117 nymphs of C. sylvestrii had been divided into 3 replicates (each replicate contained 39 nymphs) before they were exposed as 2nd instars to spores of N. locustae on Chinese cabbage leaves immersed in suspension containing 1 x 10^5 spores/µl and dried. 36.6% of the nymphs died 16 days after infection. It was found that the rest of the nymphs were not infected after examining the haemolymph. The nymphs were reinfected as 3rd instars using suspension containing 1 x 10^7 spores/µl. The remaining nymphs died 17 days after reinfection. The results are given in Table 9.

Meronts and spores were seen in the haemolymph on the 6th day after infection. Fore-, mid-, and hindgut, Malpighian tubules, and air sacs were moderately infected, fat tissue and haemolymph were heavily infected but ovaries and testes were uninfected, when examined in sections.

Even when other tissues of the nymphs carried massive infections, meronts did not spread to the gonads of either O. annulipes or C. sylvestrii and the immature eggs and sperm tubules were completely free of infection. No eggs of the latter species were laid because all infected insects died before becoming adult.

Table 8

Rate of mortality of O. annulipes infected with N. locustae

Suspension of spores applied to leaves	Nymphal instar	Days after treatment	No.of dead nymphs in 1st replicate	in 2nd replicate	in 3rd replicate	total no. of dead nymphs	% of dead nymphs
$1x10^5$sp/µl	2nd	32	11	7	9	27	16
$1x10^7$sp/µl	3rd	18	17	14	14	45	32
$1x10^9$sp/µl	5th	16	6	8	4	18	19.6
Control	2nd-5th	-	0	0	0	0	0

Table 9

Rate of mortality of C. sylvestrii infected with N. locustae

Infected dose	Nymphal instar	Days after infection	No. of dead nymphs in 1st replicate	in 2nd replicate	in 3rd replicate	total no. of dead nymphs	% of dead nymphs
$1x10^5$sp/µl	2nd	16	14	16	12	42	36.6
$1x10^7$sp/µl	3rd	17	23	24	28	75	100
Control	2nd	-	0	0	0	0	0

3.4.5 Ultrastructural study of N. locustae in fat tissue of L. migratoria

All stages lay in direct contact with the cell cytoplasm. As with the other microsporidia, meronts were limited by a simple plasma membrane and the cytoplasm contained abundant ribosomes but little endoplasmic reticulum. A few vesicles containing electron dense material were scattered in the cytoplasm (Fig.105). Nuclei were in diplokaryon arrangement and the envelope, of two unit membranes, was interupted by numerous pores (Fig.105). The unit membranes were flattened in the contact area. Division of meronts was by binary fission (Fig.106). Host mitochondria were abundant in the cytoplasm in the vicinity of meronts and sporonts, some of these appeared expanded with close packed cristae (Fig.107). There was frequent evidence of nuclear division in the meronts in the form of spindle microtubules arising from electron dense plaques, 500nm in diameter in the nuclear envelope, and condensed chromatin associated with the microtubules (Figs.105,108,109).

Sporonts had electron dense material which was deposited irregularly on the surface of the plasma membrane. They divided by binary fission (Fig.110), giving rise to 2 sporoblasts. Sporoblasts were poorly fixed: they had crenated outlines and disorganized cytoplasm (Fig.111).

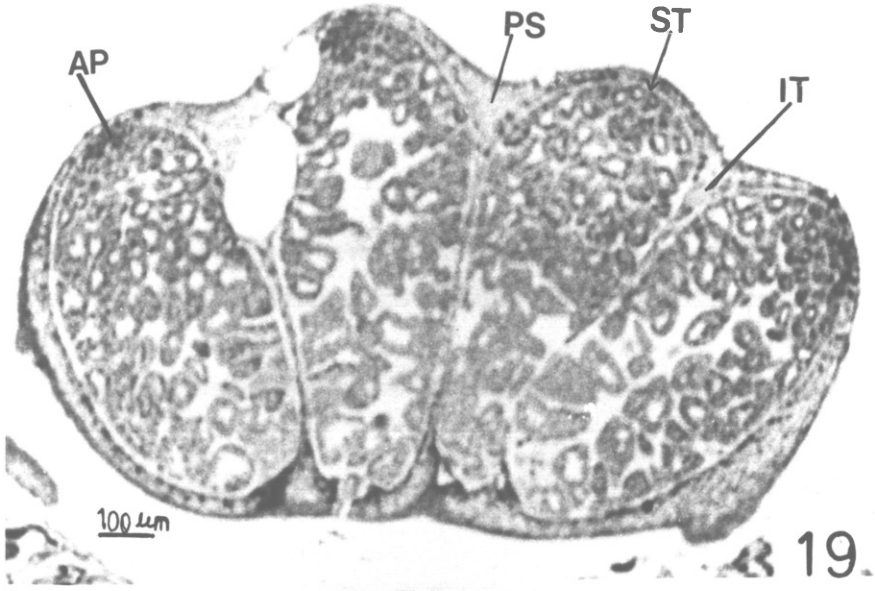
Spores had a thick wall, which consisted of a thin amorphous corrugated exospore, 50 nm thick, and an electron-lucent endospore, 250 nm thick. There were 17-19

coils of the polar tube and the angle of tilt ranged from 40^{o}-50^{o}. The tube lay in the posterior two thirds of the cell, probably adopting a single rank in mature spores (Fig.113), although some overlapping of coils was seen in developing spores (Fig.112). The 2 nuclei were found in the posterior half of the spores (Figs. 112,114).

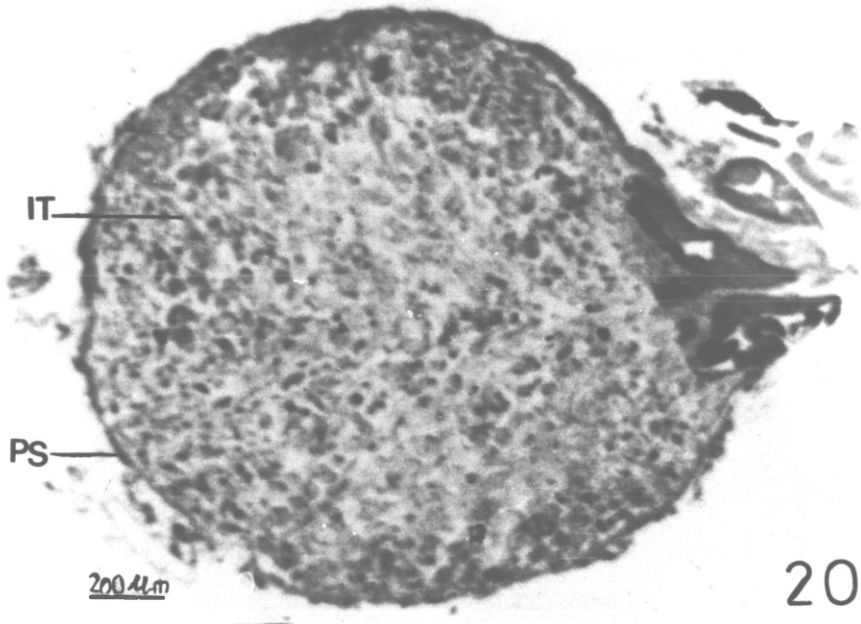
Fig. 19 : Testis of 5th instar larva of H. zea illustrating the peritoneal sheath, sperm tubule, intertubular tissue and apical cell.

Fig. 20 : L.S. of testes of adult H. zea

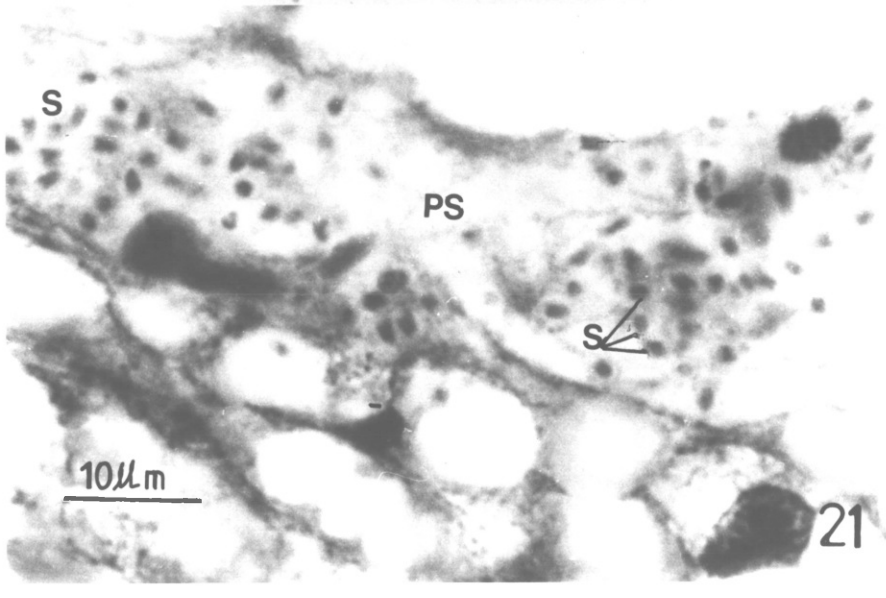
Fig. 21 : Large part of peritoneal sheath of 4th instar larval testis of H. zea infected with N. heliothidis



19.



20

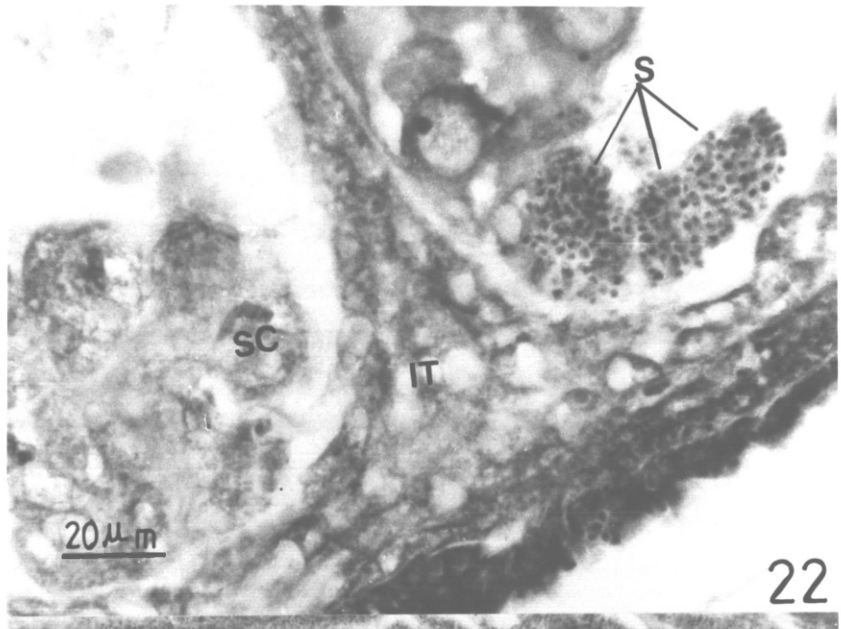


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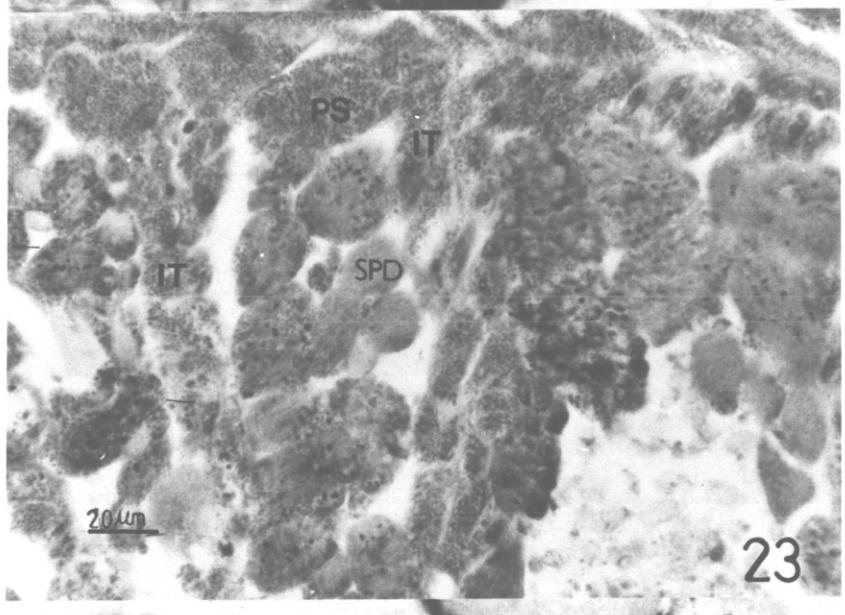
Fig. 22 : Enlarged part of testis of 5th instar larva of H. zea : Invasion of testicular cysts of sperm tubule by N. heliothidis

Fig. 23 : Section of testes of H. zea adult. Peritoneal sheath and intertubular tissue infected with N. heliothidis

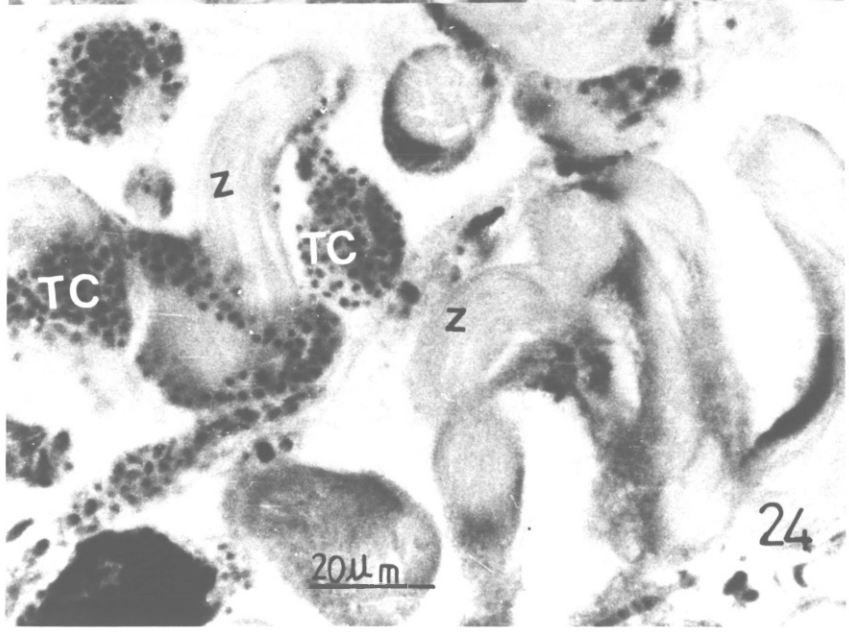
Fig. 24 : Section of testes of H. zea adult. Testicular cyst cells of spermatozoa infected with N. heliothidis



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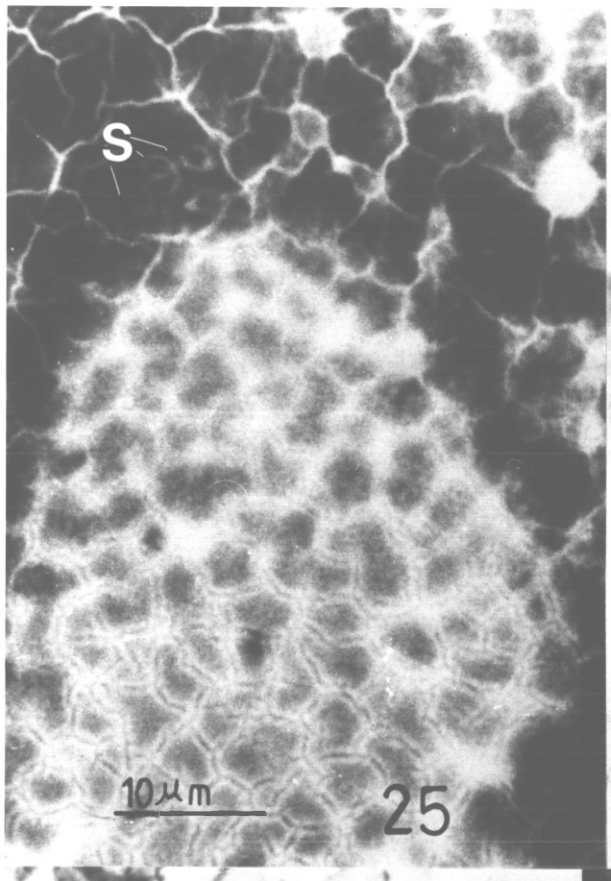
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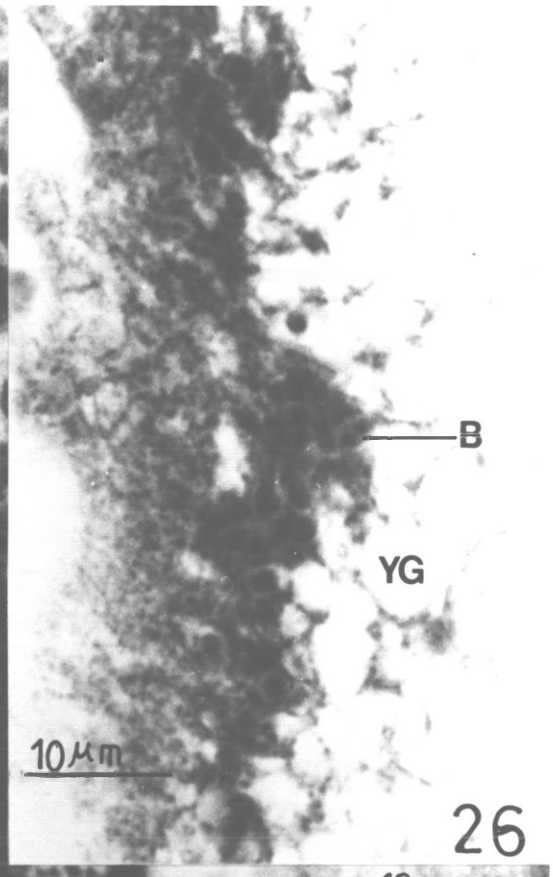
Fig. 25 : *H. zea* adult female : spores of *N. heliothidis* in a trophocyte

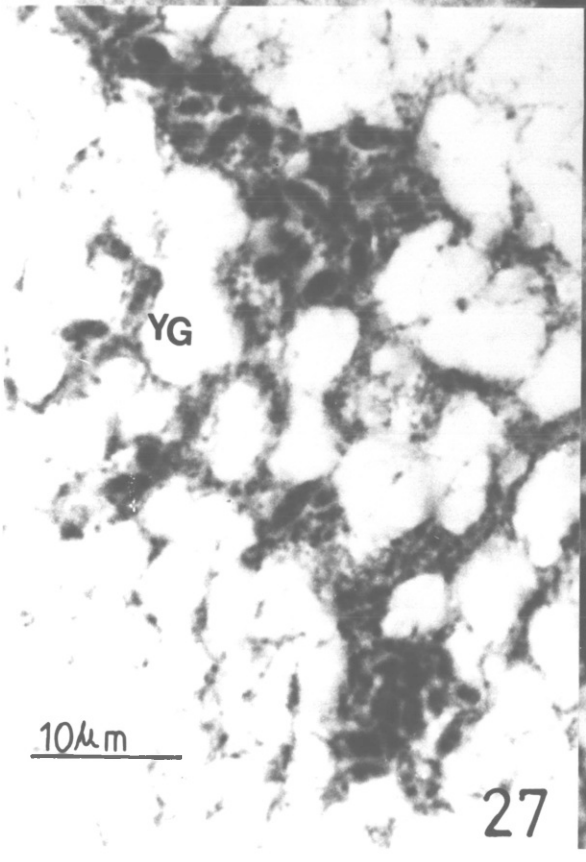
Fig. 26 : *H. zea* adult female : binucleate stages of *N. heliothidis* lying in the dense peripheral cytoplasm of an oocyte

Fig. 27 : *H. zea* adult female : binucleate stages of *N. heliothidis* clumped in the centre of an oocyte

Fig. 28 : *H. zea* : enlarged part of trophocyte : binucleate stages of *N. heliothidis* among the remains of the trophocyte


S
10µm
25


B
YG
10µm
26


YG
10µm
27

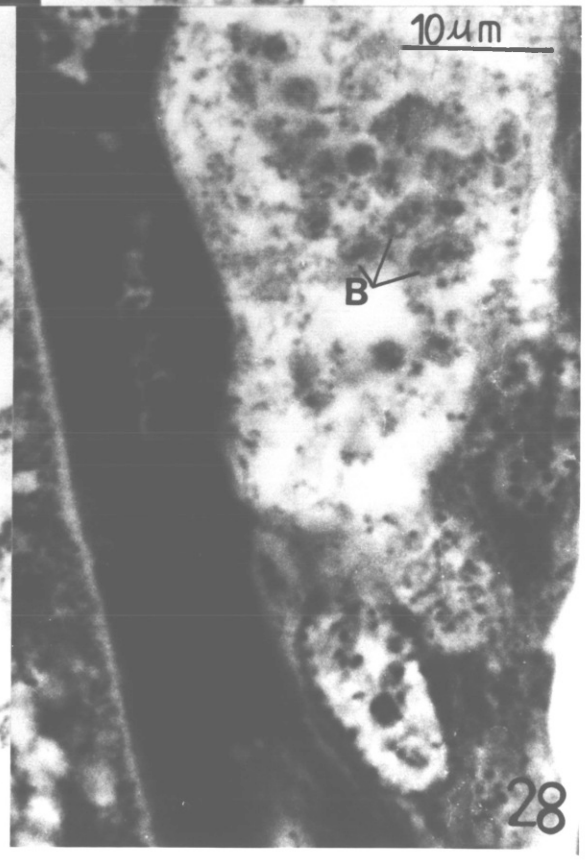

10µm
B
28

Fig. 29 : Meront of N. heliothidis in an oocyte of adult female H. zea

Fig. 30 : Early sporont of N. heliothidis in an oocyte of adult female H. zea : Some deposition of surface coat material on plasma membrane (arrows)

Fig. 31 : Early sporont of N. heliothidis in the trophocyte of adult female H. zea

Fig. 32 : Early sporont of N. heliothidis in interfollicular tissue of adult female H. zea

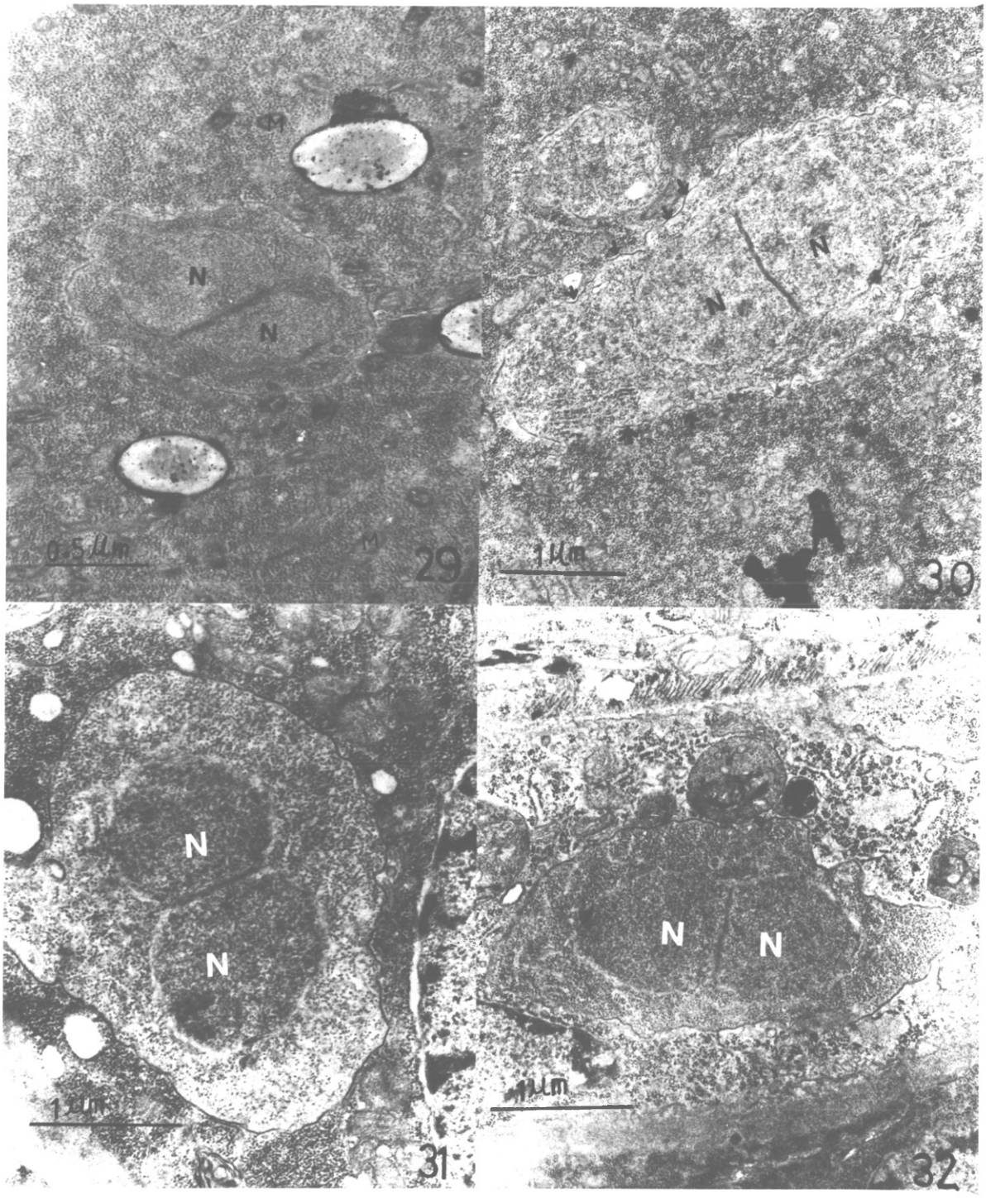

N
N
0.5 µm
29
N
N
1 µm
30
N
N
1 µm
31
N
N
1 µm
32

Fig. 33 : Late sporont of N. heliothidis in mature oocyte of adult female H. zea

Fig. 34 : Sporoblast of N. heliothidis in the trophocyte of adult female H. zea

Fig. 35 : Sporoblasts of N. heliothidis in mature oocyte of adult female H. zea

Fig. 36 : Degenerate spore of N. heliothidis in the interfollicular tissue of adult female H. zea

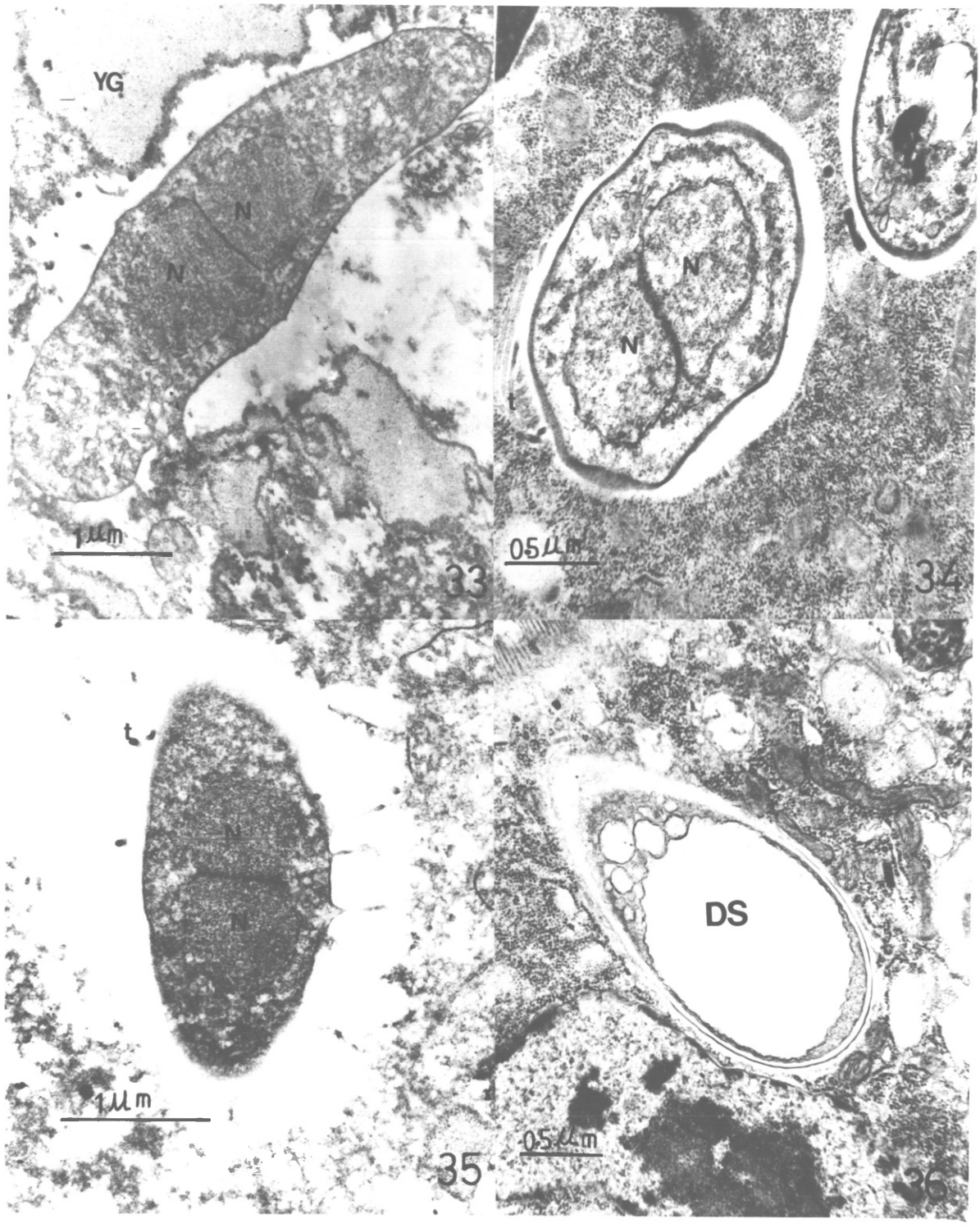
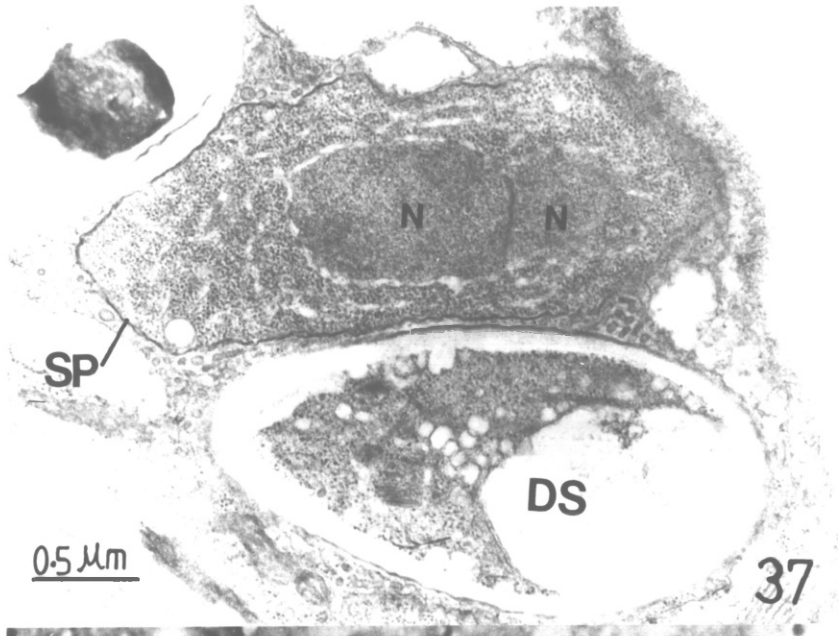

YG
N
N
1µm
33
N
N
t
0.5µm
34
t
N
N
1µm
35
DS
0.5µm
36

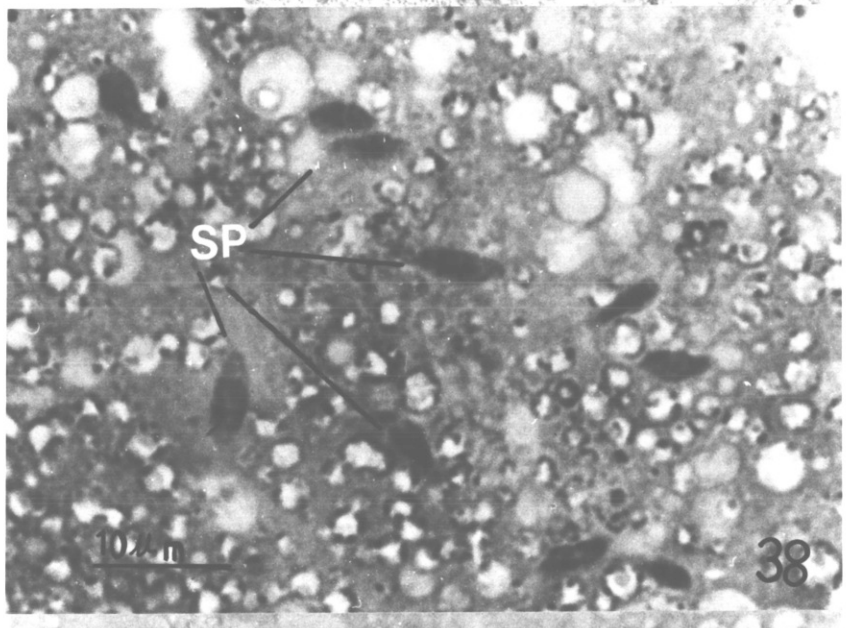
Fig. 37 : Epithelial sheath of an ovariole of adult female H. zea invaded by N. heliothidis

Fig. 38 : Binucleate sporonts of N. heliothidis in egg smear of H. zea

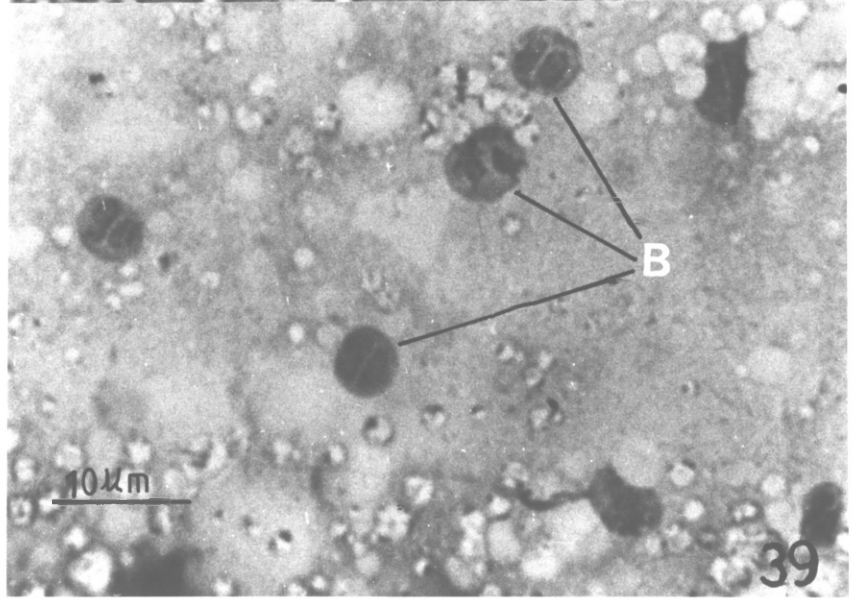
Fig. 39 : Binucleate stages of N. heliothidis in egg smear of H. zea



37



38



39

Fig. 40 : Small pockets of binucleate stages of N. heliothidis around the periphery in a section of non-embryonated eggs of female H. zea

Fig. 41 : Binculeate stages of N. heliothidis scattered throughout section of a non-embryonated egg of H. zea

Fig. 42 : Binculeate stages of N. heliothidis underneath the serosa in a section of an embryonated egg of H. zea

Fig. 43 : Single spore of N. heliothidis in a nerve ganglia in a fully developed larva from an embryonated egg of H. zea (It looks as if it is not a genuine infection)

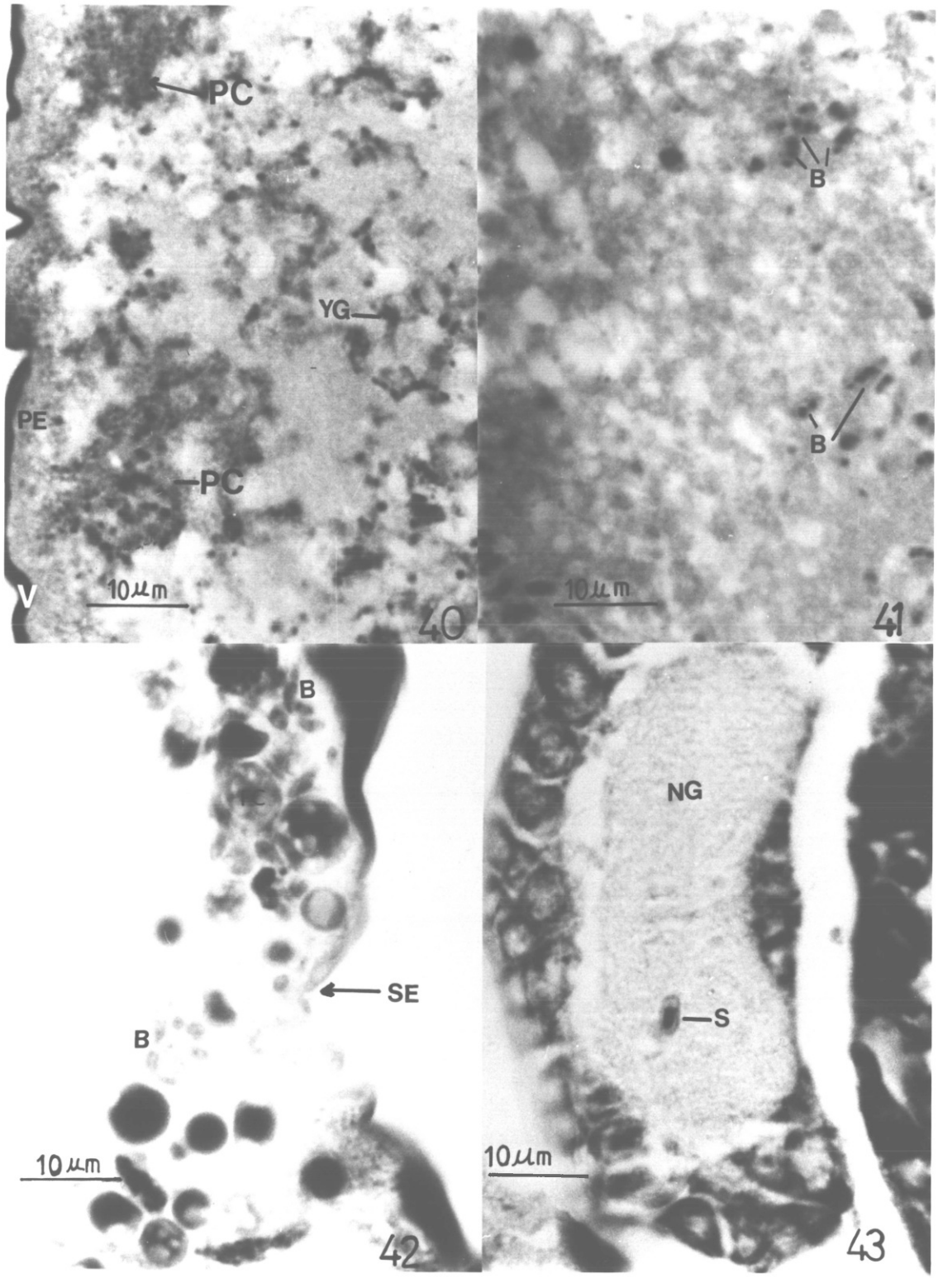
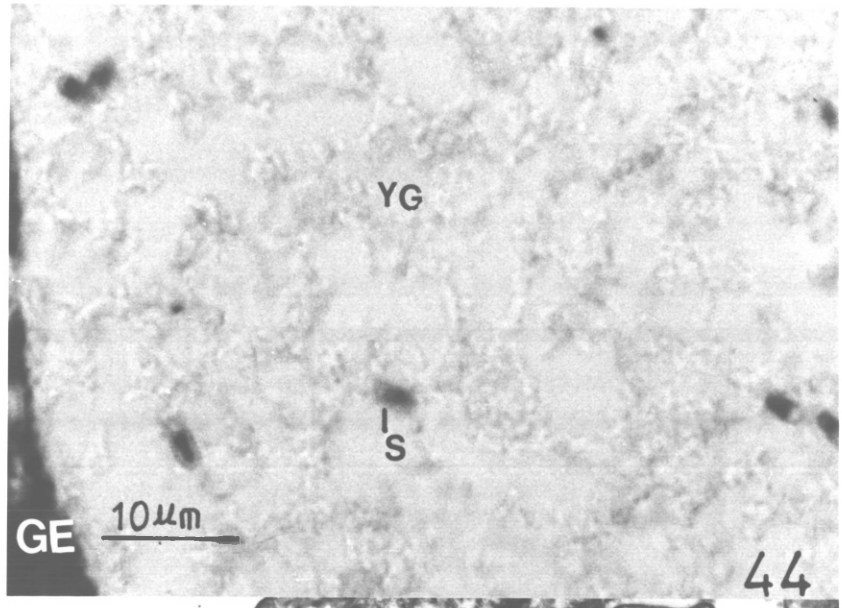

PC
YG
PE
PC
V
10 µm
40
B
B
10 µm
41
B
SE
B
10 µm
42
NG
S
10 µm
43

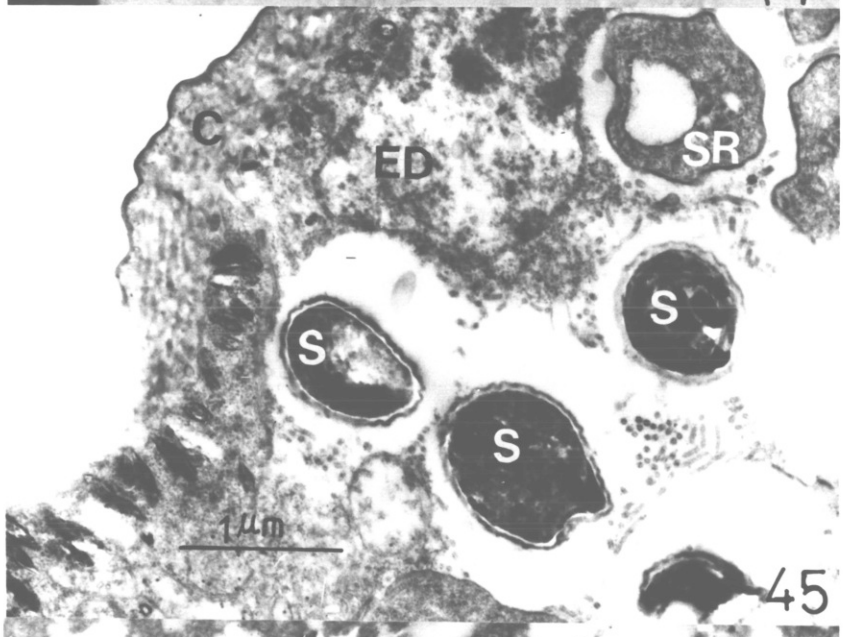
Fig. 44 : Spores of N. heliothidis in the yolk in the gut lumen of a fully developed larva from an embryonated egg of H. zea

Fig. 45 : Spores and sporogonic stages of N. heliothidis infecting the epidermis of a fully developed larva from an embryonated egg of H. zea

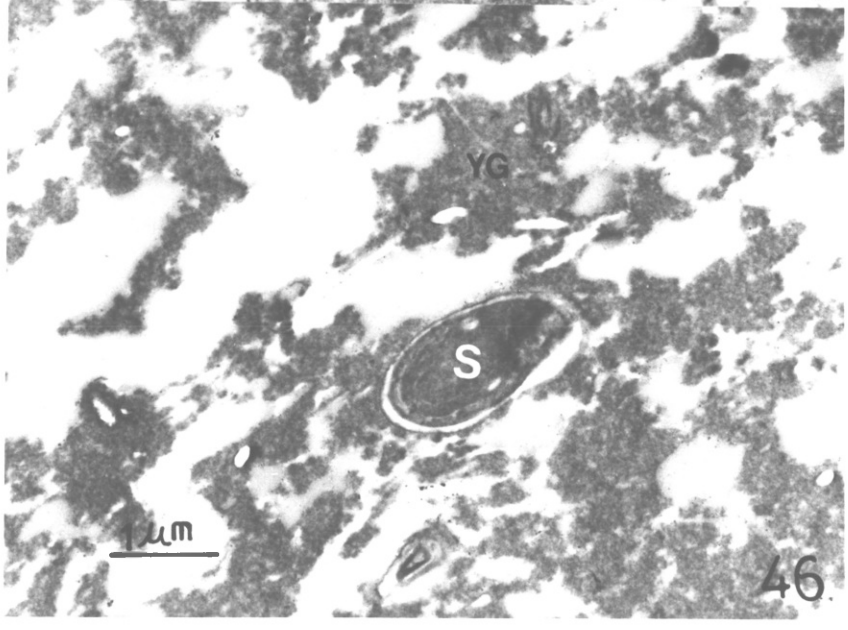
Fig. 46 : Spore among the yolk granules in the gut lumen of a fully developed larva from an embryonated egg of H. zea



44



45

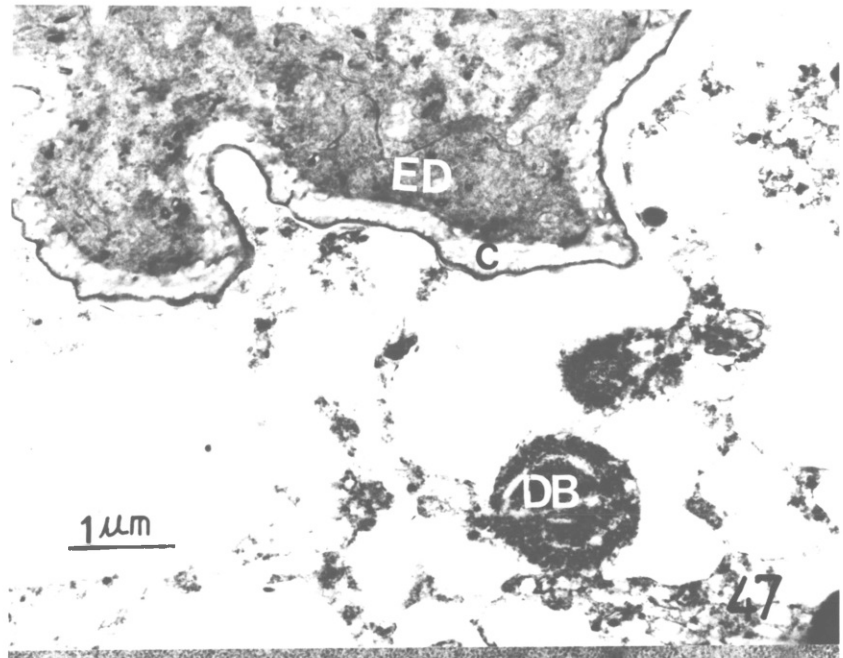


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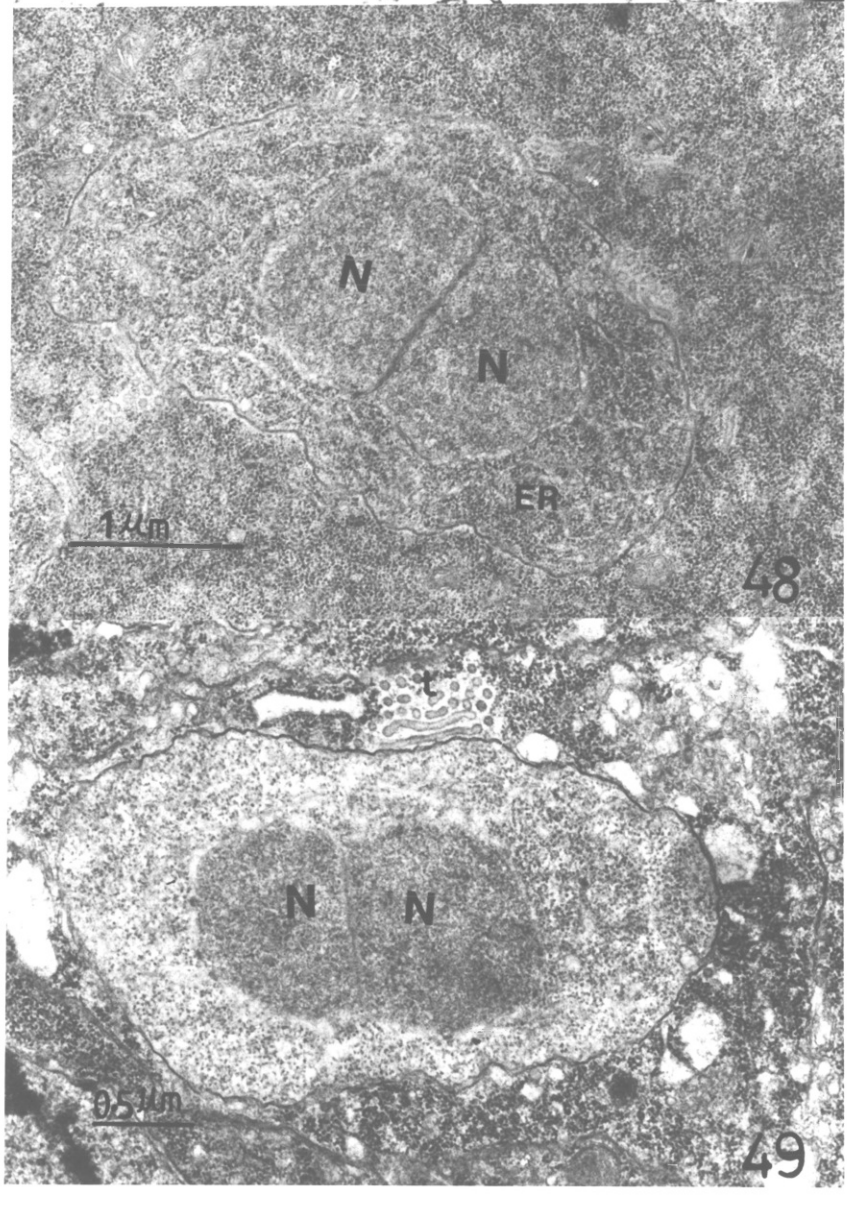
ig. 47 : Degenerate binculeate stage of N. heliothidis outside embryonic tissue, in an embryonated egg of H. zea

Fig. 48 : Meront of N. heliothidis showing endoplasmic reticulum

Fig. 49 : Meront of N. heliothidis showing cytoplasm less dense than nucleoplasm. Some tubules were present outside the plasma membrane



47



48

49

Fig. 50 : Meront of N. heliothidis just after cytoplasmic division

Fig. 51 : Numerous mitochondria around meront of N. heliothidis

Fig. 52 : Early sporont of N. heliothidis showing difference in surface membrane organization

Fig. 53 : Sporont of N. heliothidis showing 20 nm diameter tubules in a space around the parasite

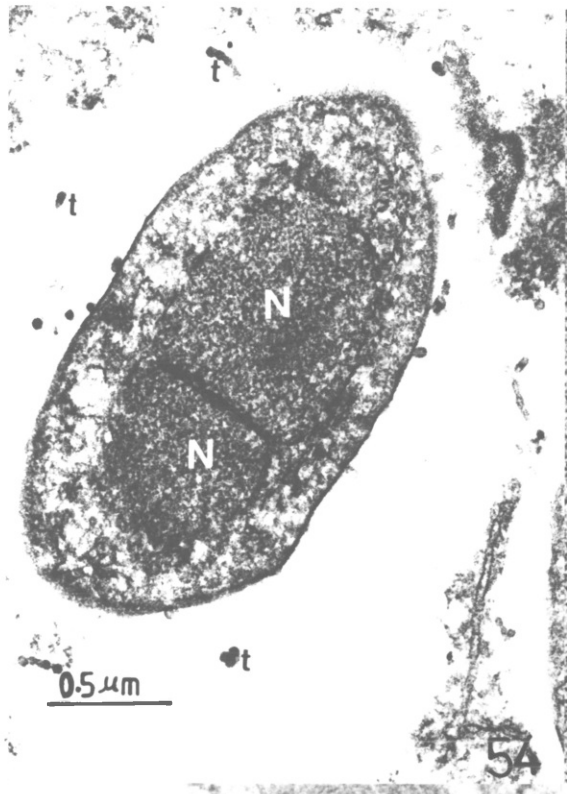

N
N
N
N
1µm
50
M
05 µm
51
N
1 µm
52
N
N
1µm
53

Fig. 54 : Early sporoblast of N. heliothidis showing 20 nm diameter tubules in a space around the parasite

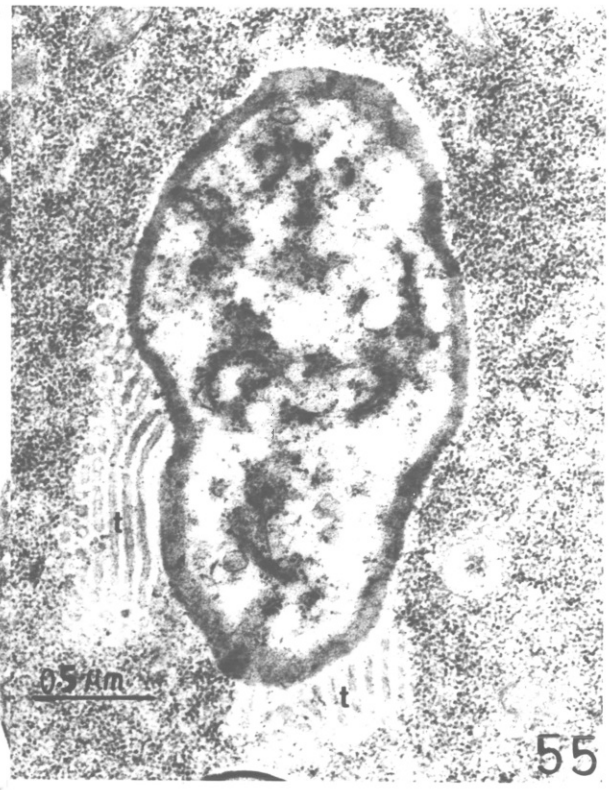
Fig. 55 : Late sporoblast of N. heliothidis showing tubules in association with its surface

Fig. 56 : Mature spore of N. heliothidis with the polaroplast and the straight basal part of the polar tube and the most anterior coil

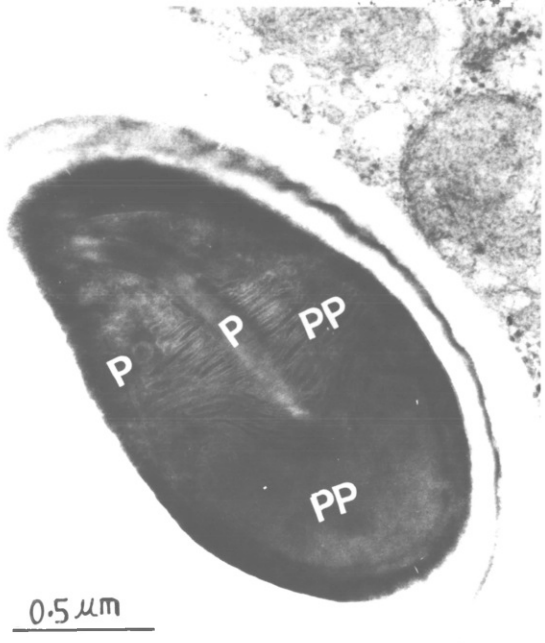
Fig. 57 : Mature spore of N. heliothidis showing 10 polar tube coils



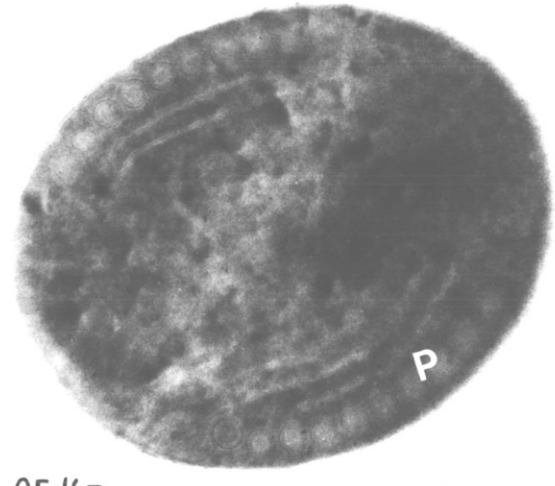
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55



56

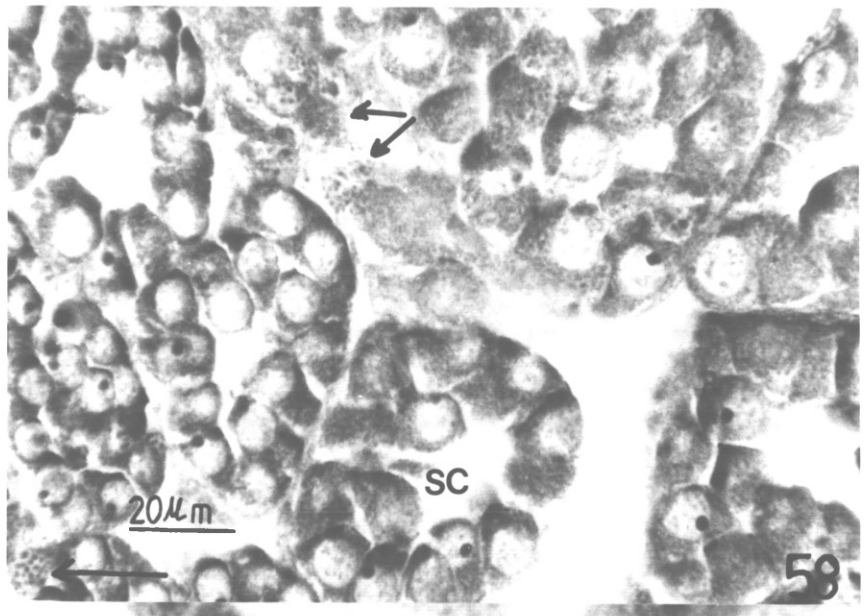


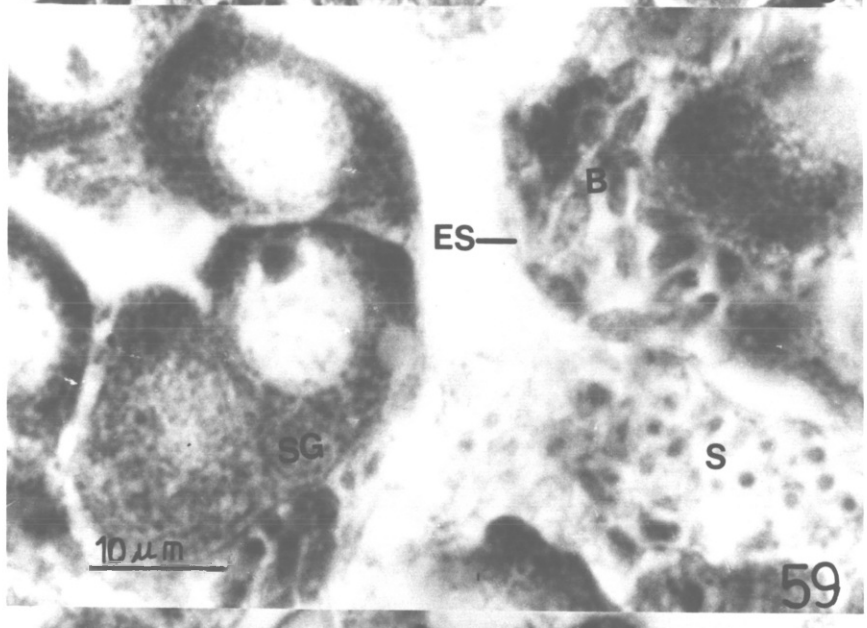
57

Fig. 58 : Several testicular cysts in the testis of 5th instar H. zea, showing the enclosing sheath of a testicular cyst (double arrows) and spermatogonia (single arrow) infected with N. bombycis spores

Fig. 59 : Enlarged part of testicular cysts of a sperm tubule of 5th instar larvae of H. zea showing binucleate stages and spores of N. bombycis infecting the enclosing sheath of the testicular cysts

Fig. 60 : The testicular cyst cells limiting the spermatids of testes of adult H. zea infected with N. bombycis (arrows)


SC
20µm
58


B
ES
SG
S
10µm
59

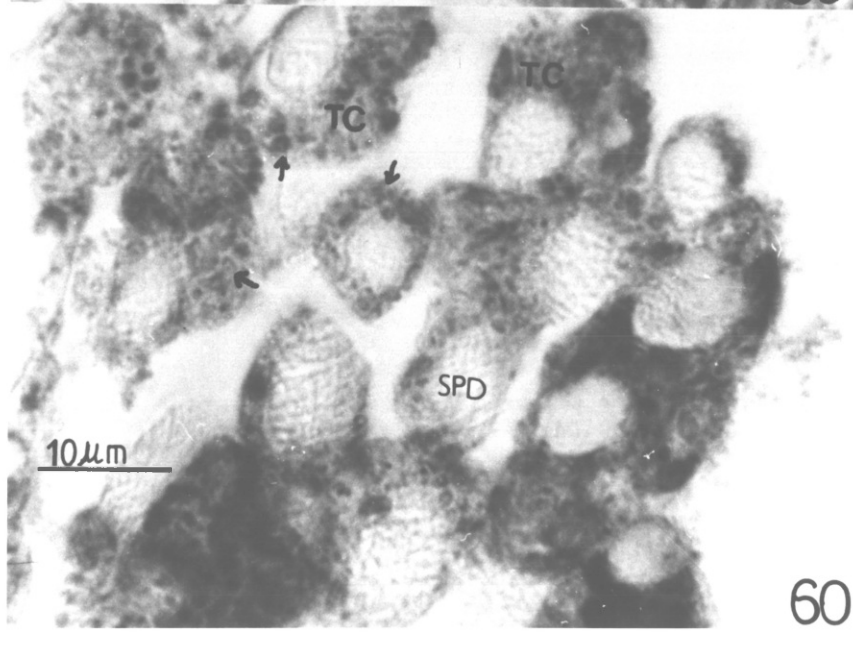
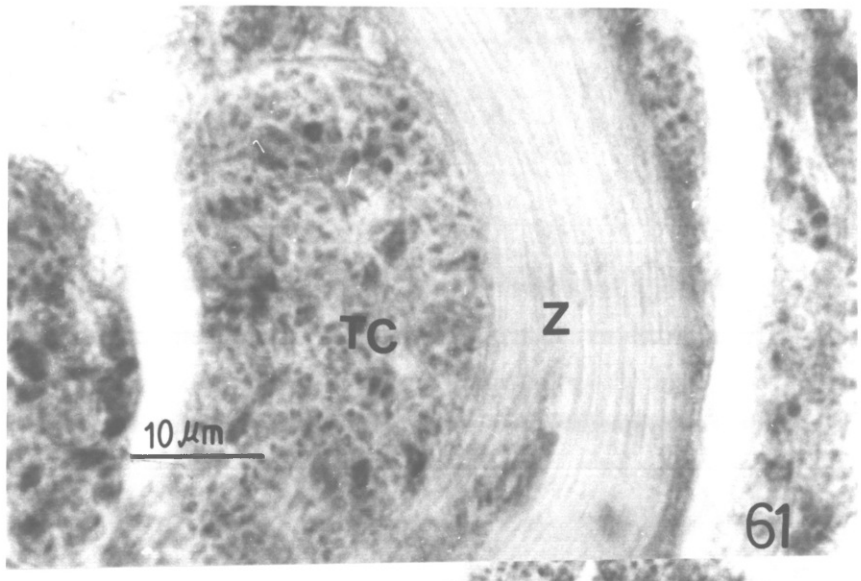

TC
TC
SPD
10µm
60

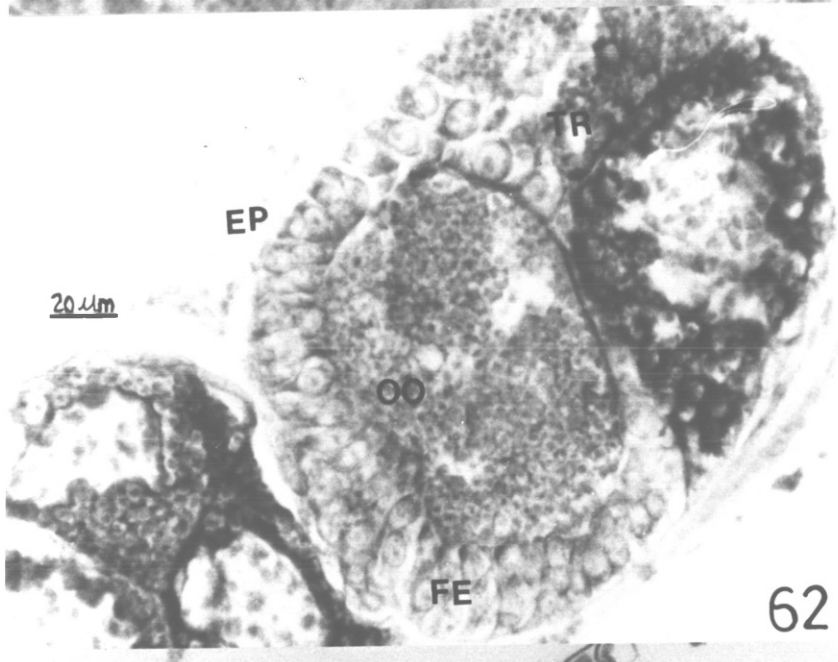
Fig. 61 : Testicular cyst cells of spermatozoa of testes of adult H. zea invaded by N. bombycis

Fig. 62 : H. zea : heavily infected follicle with multilayered follicular epithelium. Trophocytes, oocytes and epithelial cells infected with N. bombycis

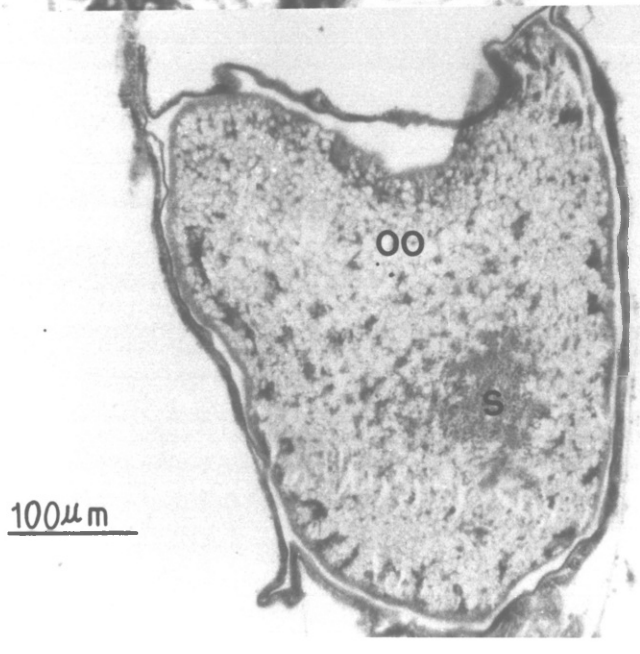
Fig. 63 : Mature oocyte of H. zea lightly infected with N. bombycis in the centre of the oocyte only



61



62

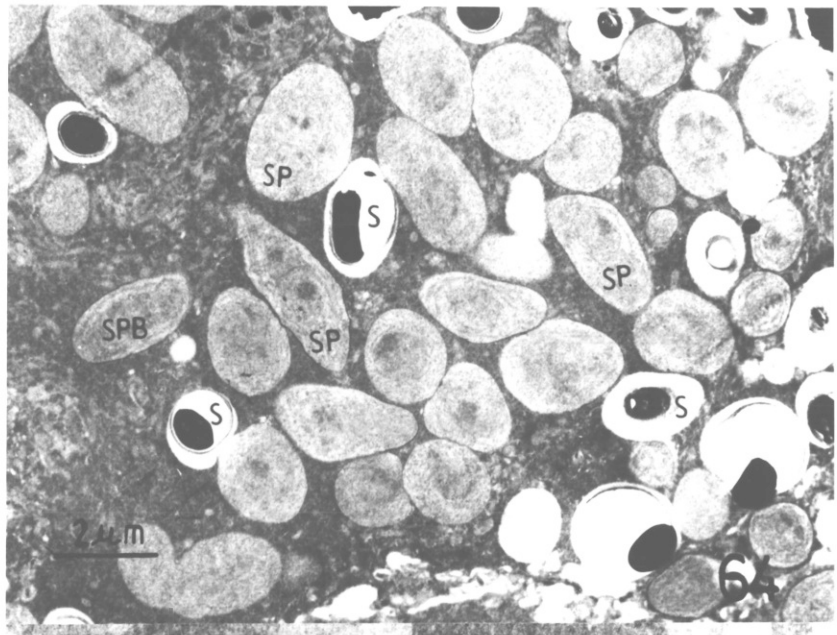


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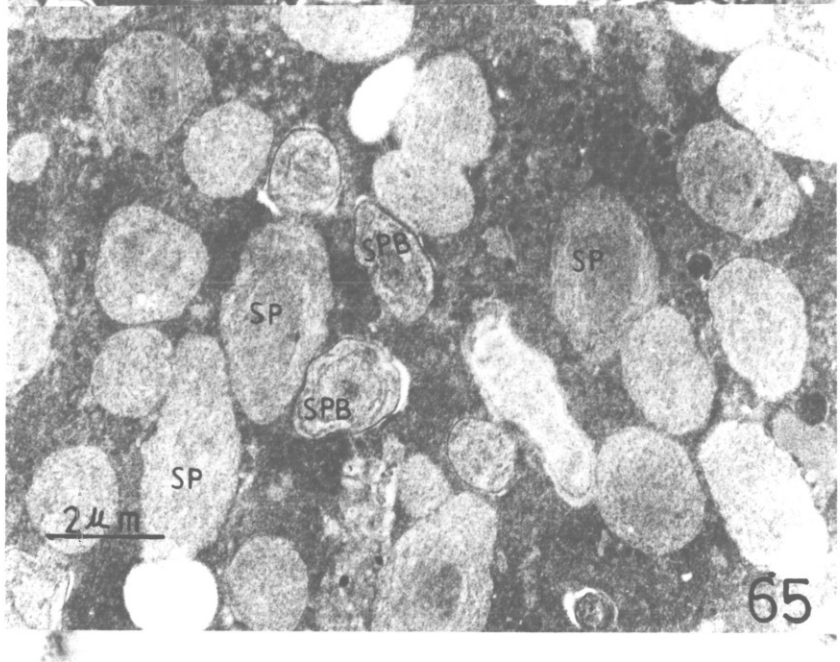
Fig. 64 : Meronts, sporonts, sporoblasts and spores of N. bombycis in immature oocyte of H. zea

Fig. 65 : Meronts, sporonts and sporoblasts of N. bombycis in trophocyte of immature follicle of H. zea

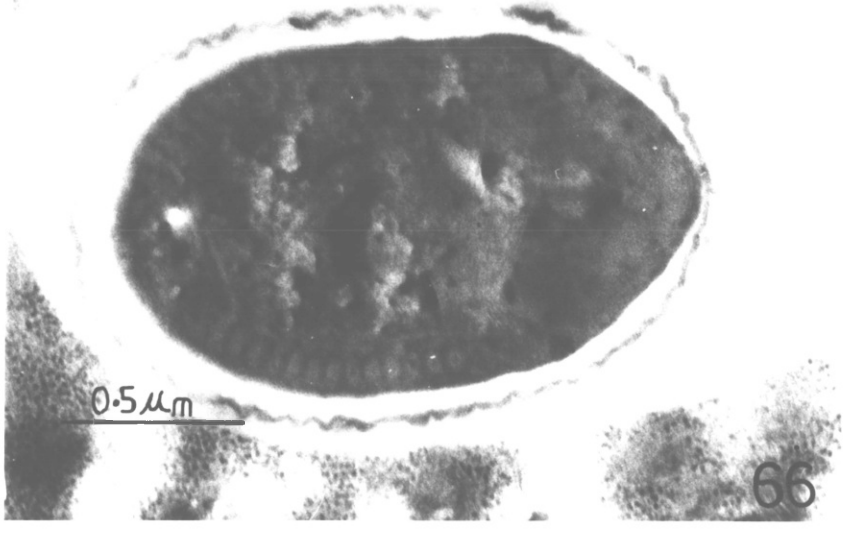
Fig. 66 : Well developed spore of N. bombycis in the ovarial tissue of H. zea



64



65



66

Fig. 67 : Degenerate spores of N. bombycis in interfollicular tissue of H. zea

Fig. 68 : Stages of N. bombycis in the follicular epithelium of H. zea

Fig. 69 : Merogonic and sporogonic stages of N. bombycis in the epithelial sheath of H. zea

Fig. 70 : Meront of N. bombycis with 2 nuclei in diplokaryon arrangement, each nucleus has an electron dense material resembling chromosomes

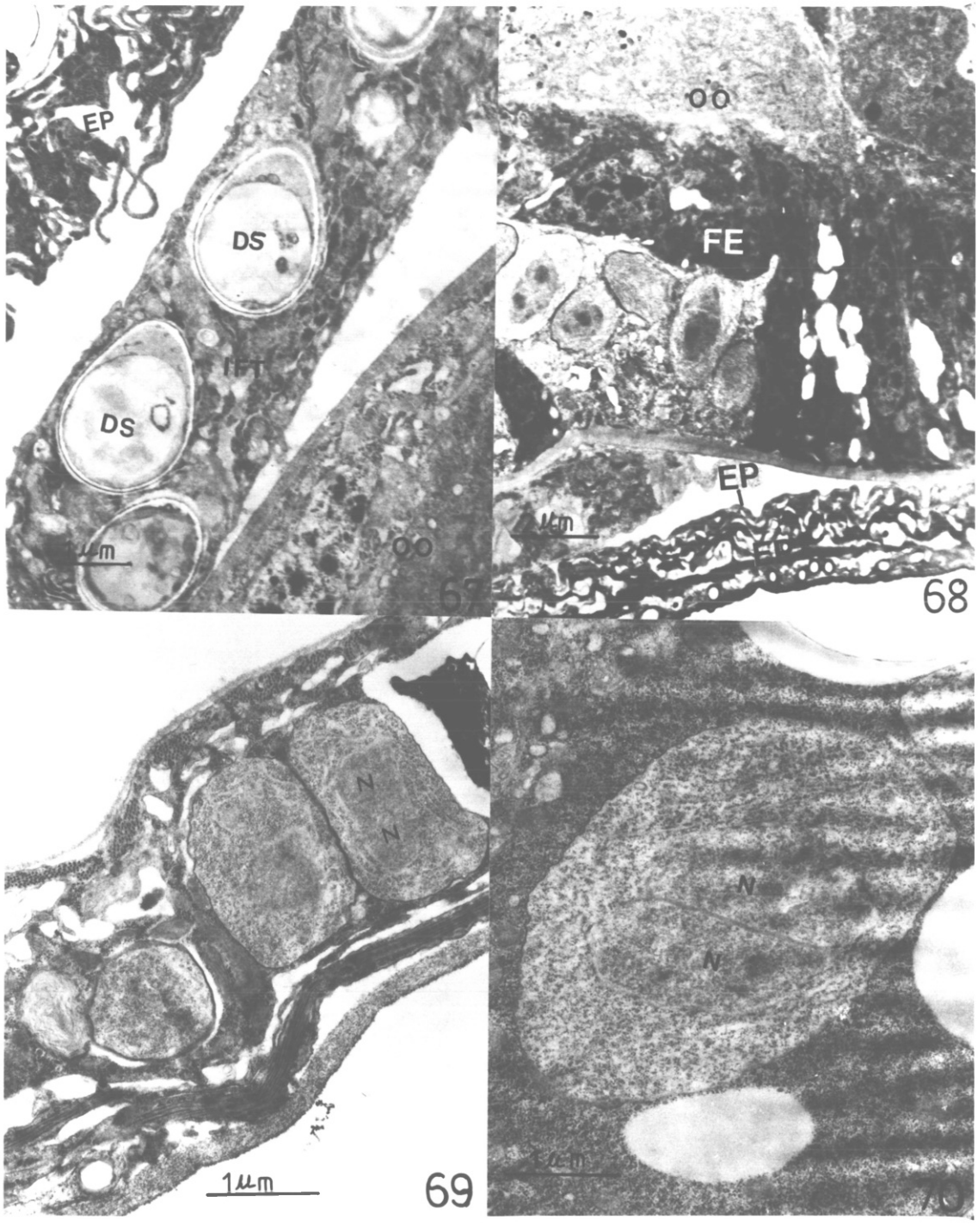

EP
DS
DS
TET
OO
1µm
67
OO
FE
EP
1µm
68
N
N
1µm
69
N
N
70

Fig. 71 : Meront of N. bombycis dividing by binary fission

Fig. 72 : Sporont of N. bombycis with electron-dense material external to the plasma membrane deposited from the poles towards the centre

Fig. 73 : Division of sporont of N. bombycis into 2 sporoblasts

Fig. 74 : Early sporoblast of N. bombycis with endoplasmic reticulum, a section of the polar tube and Golgi apparatus

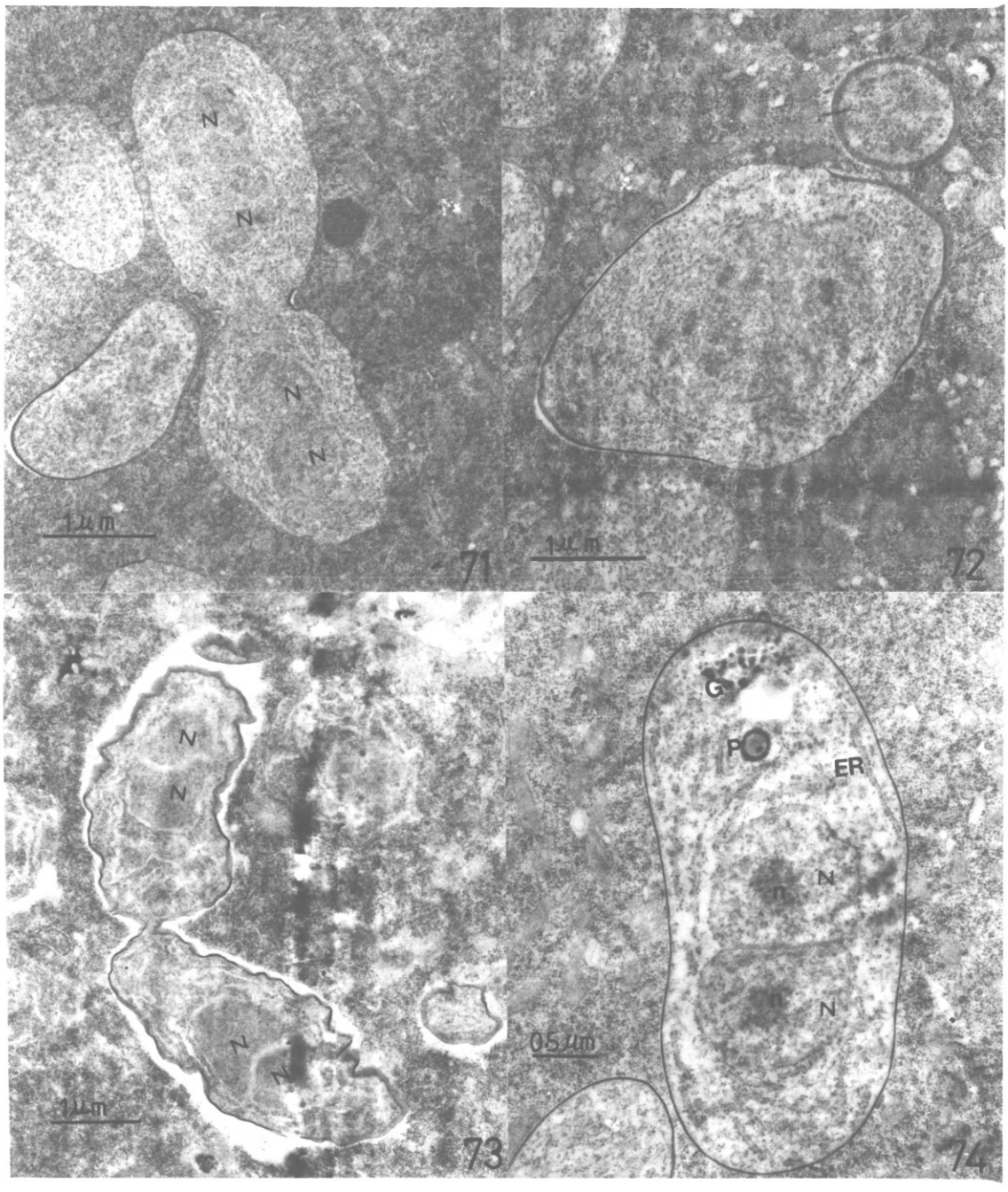

N
N
N
N
1 µm
71
1 µm
72
N
N
N
N
1 µm
73
G
P
ER
N
N
0.5 µm
74

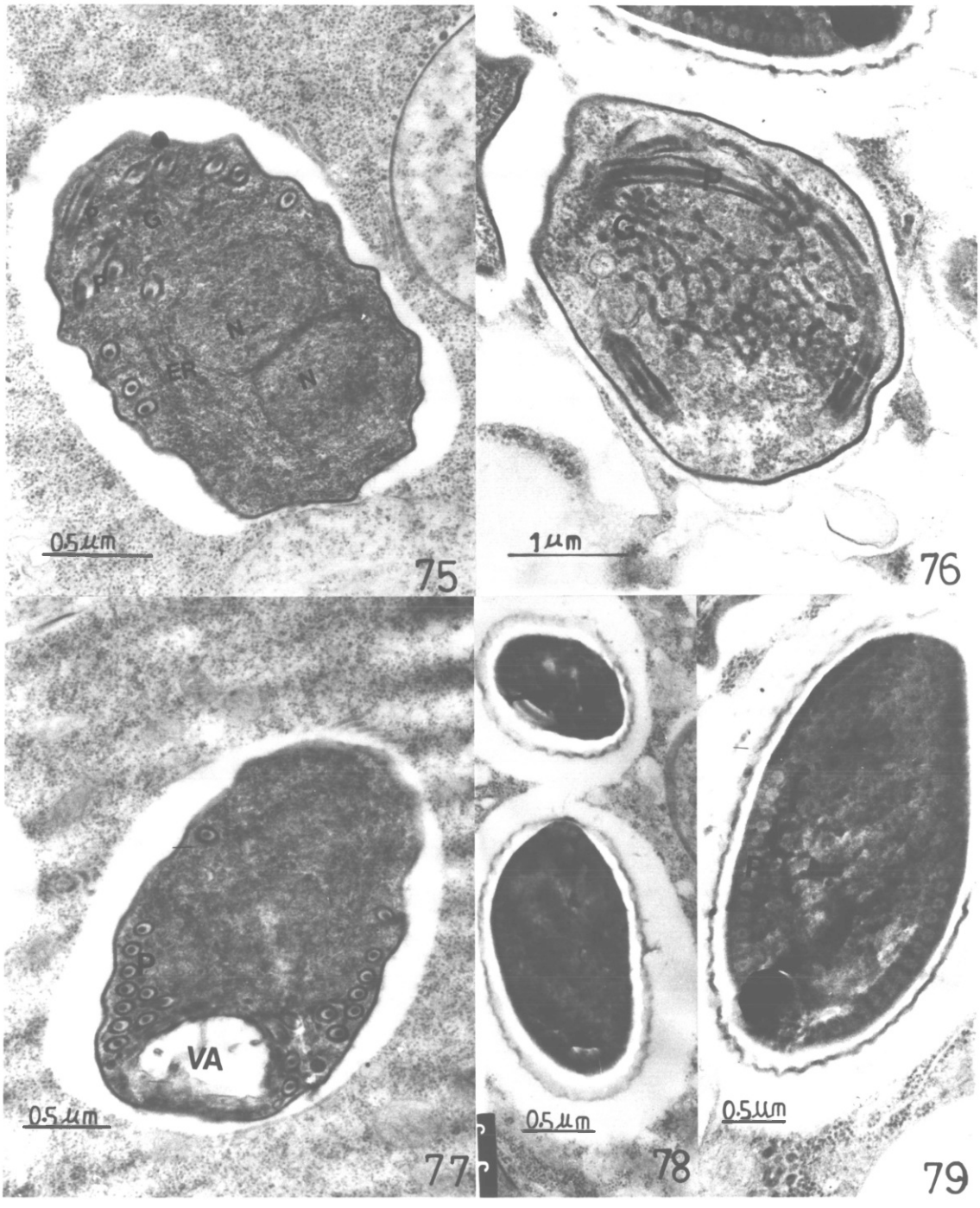
Fig. 75 : Sporoblast of N. bombycis with transverse and longitudinal sections of the polar tube

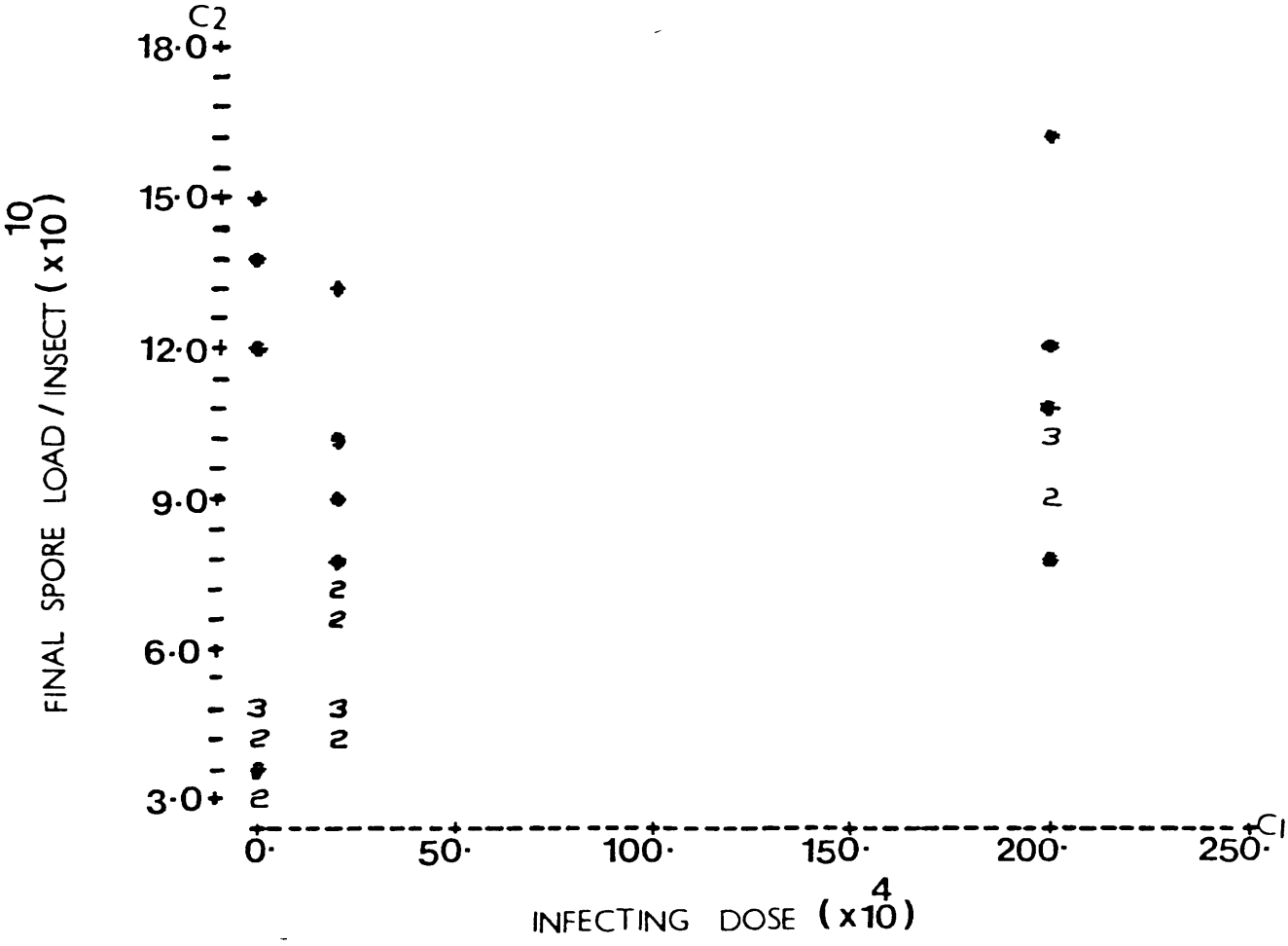
Fig. 76 : Sporobalst of N. bombycis with longitudinal section of polar tube and Golgi apparatus

Fig. 77 : Late sporobalst of N. bombycis with transverse sections of polar tube and posterior vacuole

Fig. 78 : Longitudinal section of N. bombycis spore with endospore thinned to 20 nm at the anterior end

Fig. 79 : Spore of N. bombycis with 13 - 14 coils of the polar tube in a single rank


0.5µm
75
1µm
76
VA
0.5µm
77
0.5µm
78
0.5µm
79



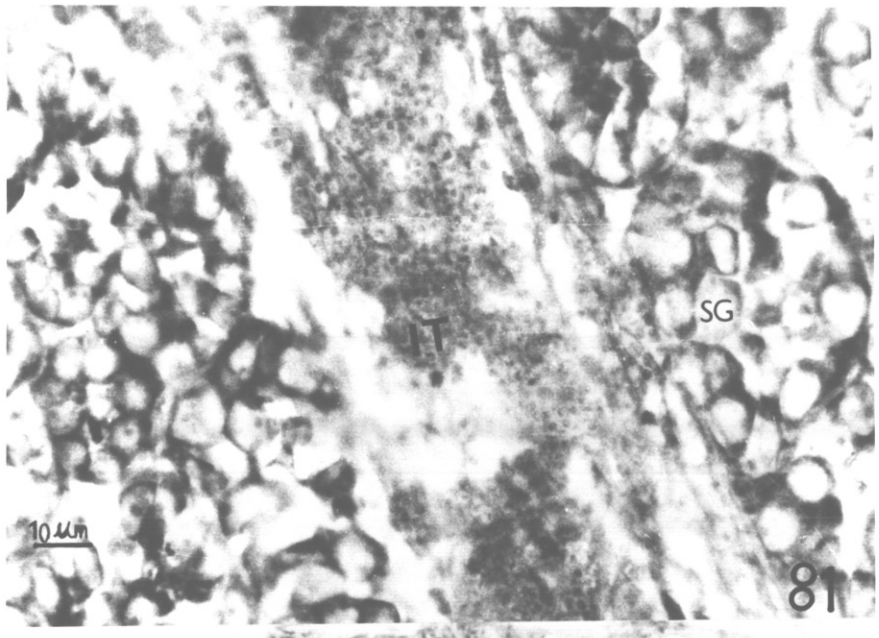
FIG·80

Relation between infected dose and spore of P. operophterae harvested from H. zea

Fig. 81 : Enlarged part of testis of 5th instar larva of H. zea showing intertubular tissue infected with spores of P. operophterae

Fig. 82 : Peritoneal sheath of testes of adult H. zea infected with spores of P. operophterae

Fig. 83 : Intertubular tissue and testicular cyst cells of testes of adult H. zea, infected with spores and developmental stages of P. operophterae


IT
SG
10 µm
81


PS
20 µm
82

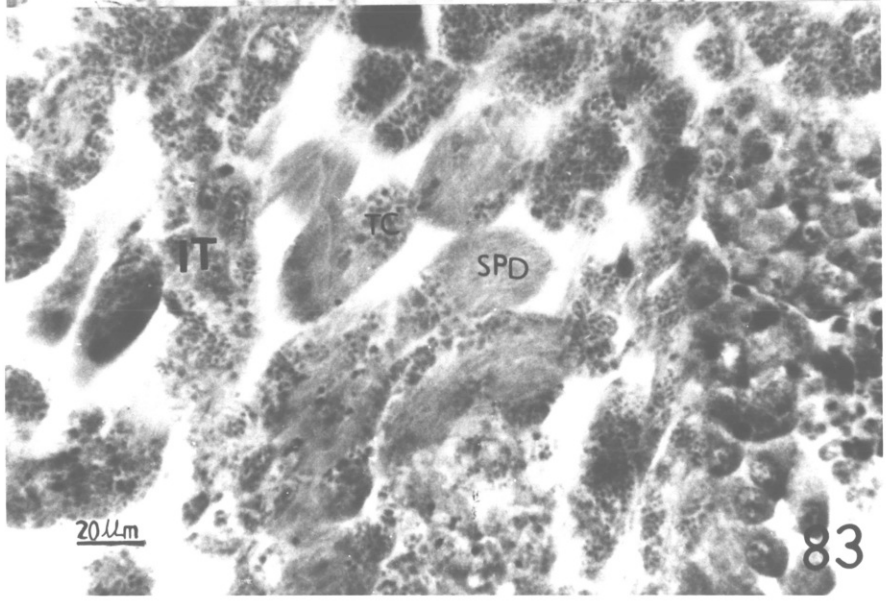
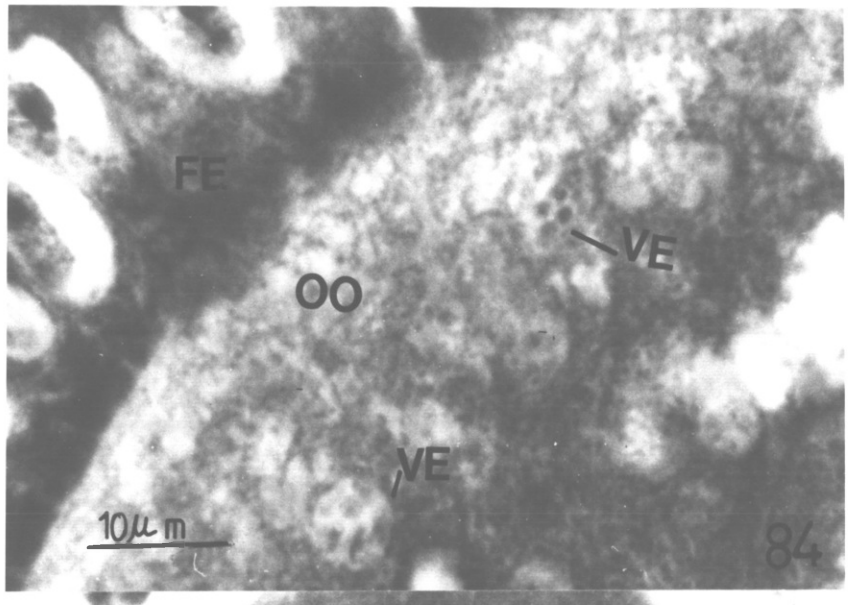

IT
TC
SPD
20 µm
83

Fig. 84 : Vesicles in the oocyte of H. zea, containing multiple stages of P. operophterae (arrows point to sporophorous vesicles)

Fig. 85 : Vesicles in a trophocyte of H. zea, containing stages of P. operophterae (arrows point to sporophorous vesicles)

Fig. 86 : Meront and isolated spores of P. operophterae in oocyte of H. zea


FE
OO
VE
VE
10µm
84


VE
VE
10µm
85

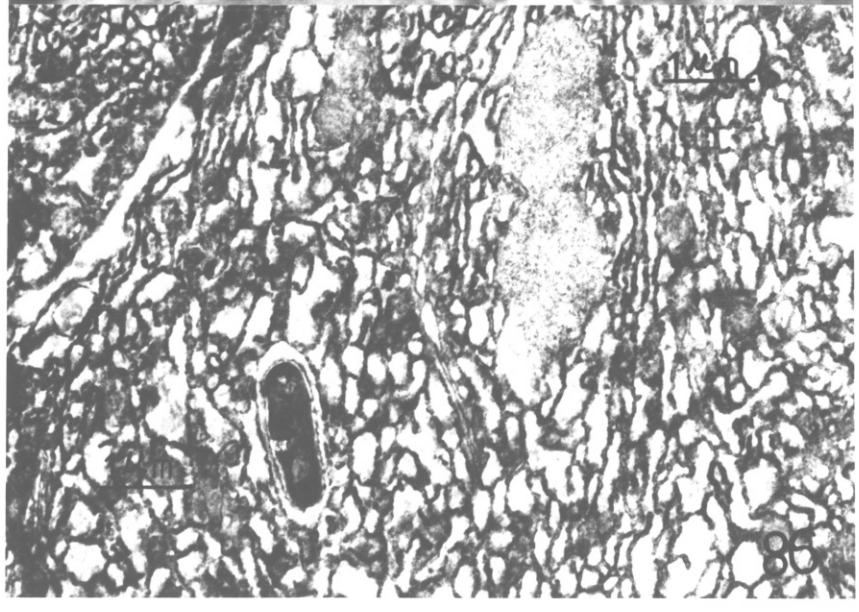

86

Fig. 87 : Sporophorous vesicle containing sporogonic stages of P. operophterae in oocyte of H. zea. Earlier developmental stages (probably also sporogonic) are present close by

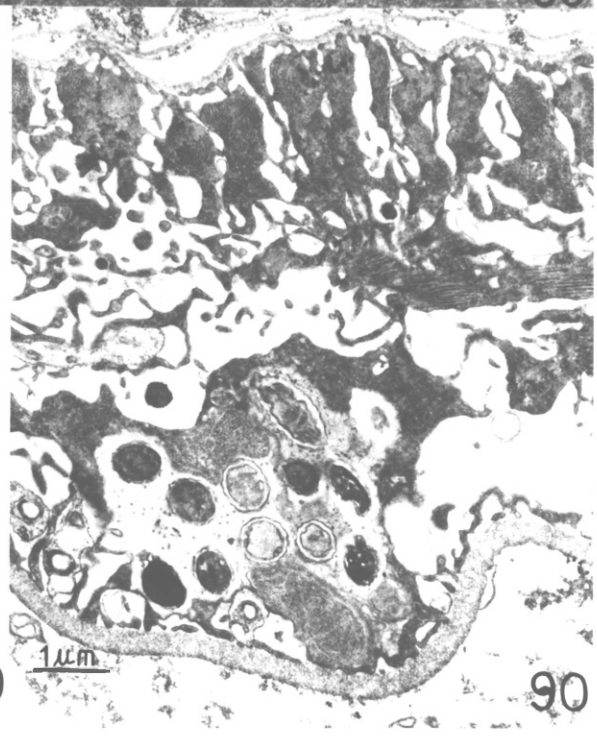
Fig. 88 : Sporophorous vesicle of P. operophterae in a trophocyte of H. zea

Fig. 89 : Meronts and a spore of P. operophterae in separate vesicles in interfollicular tissue of H. zea

Fig. 90 : Spores and sporoblasts of P. operophterae in a large vesicle in the epithelial sheath of H. zea


1µm
88


1µm
89


1µm
90

Figs. 91 - 98 : Stages of P. operophterae in (OVARIAL TISSUE) of H. zea

Fig. 91 : Uninucleate meront surrounded by plasma membrane and lying within a close-fitting vacuolar membrane

Fig. 92 : Meront during division, when the vacuolar membrane divides with the parasite

Fig. 93 : Early sporont contracting away from the vacuolar membrane. The electron dense surface coat has been added in patches (arrows).

Fig. 94 : Irregular sporont : the electron dense structures in the nucleus may be chromosomes. The surface coat can be seen in places (arrows)

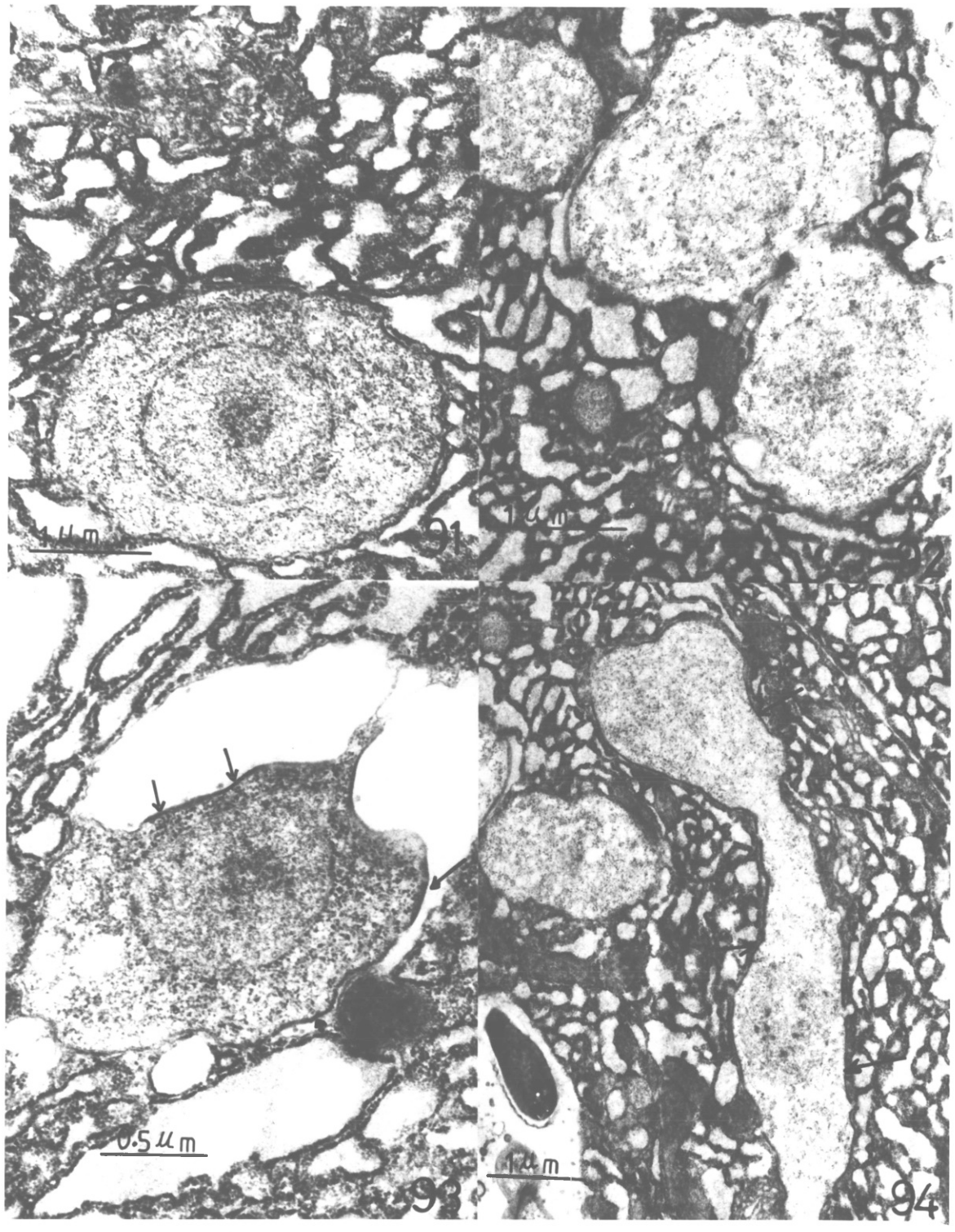

1 µm
91
1 µm
0.5 µm
93
1 µm
94

Fig. 95 : Dividing sporont : the vacuole is clearly seen between the division products

Fig. 96 : Meront or early sporont : the vacuolar membrane is still close fitting but the surface coat is deposited in patches (arrows)

Fig. 97 : Vacuole contained sporoblasts and spores : a fine matrix containing granules is present in the vacuole

Fig. 98 : Vacuole of P. operophterae with late sporoblasts showing sections of the polar tube : prominent granules are present in the vacuole

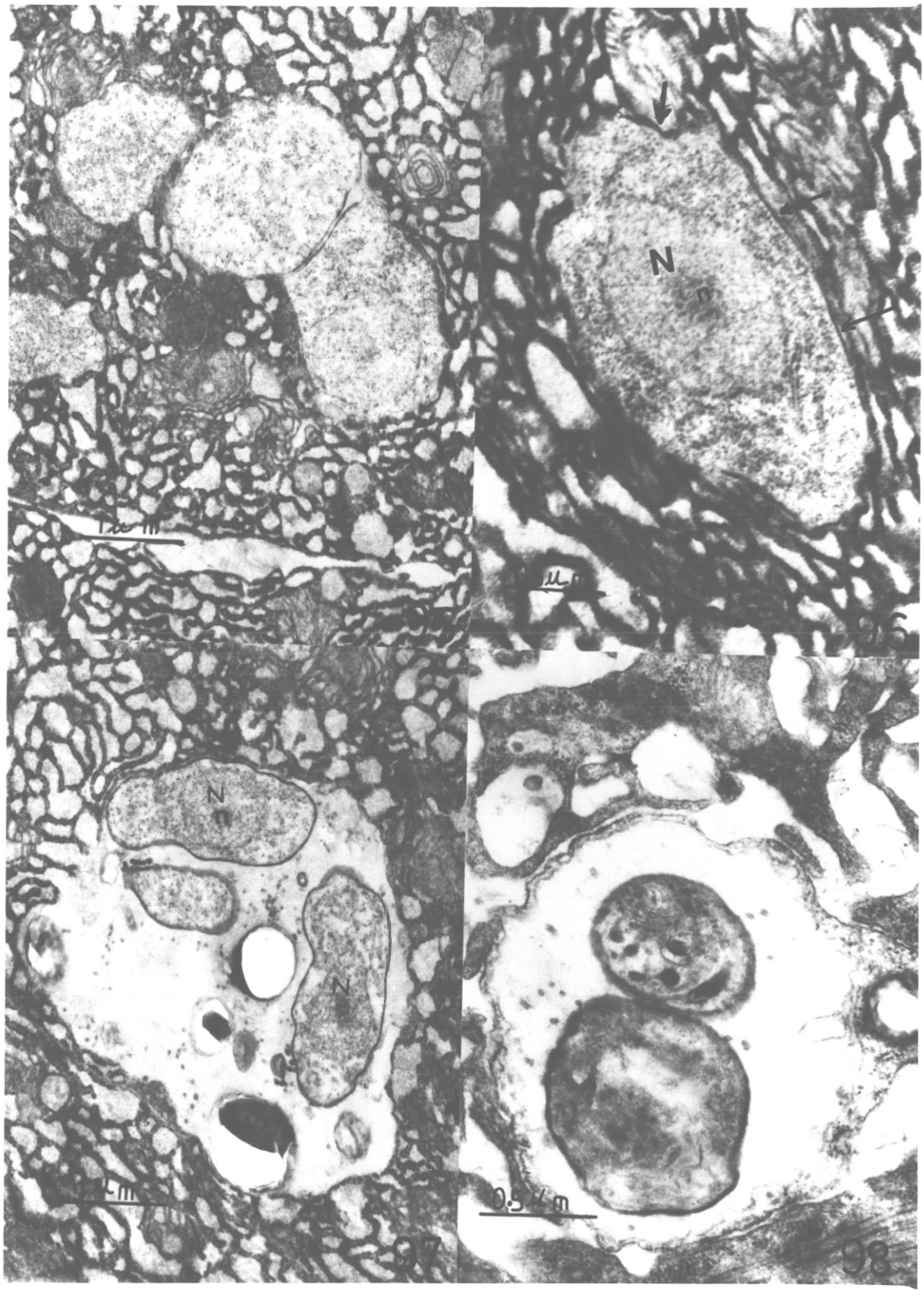
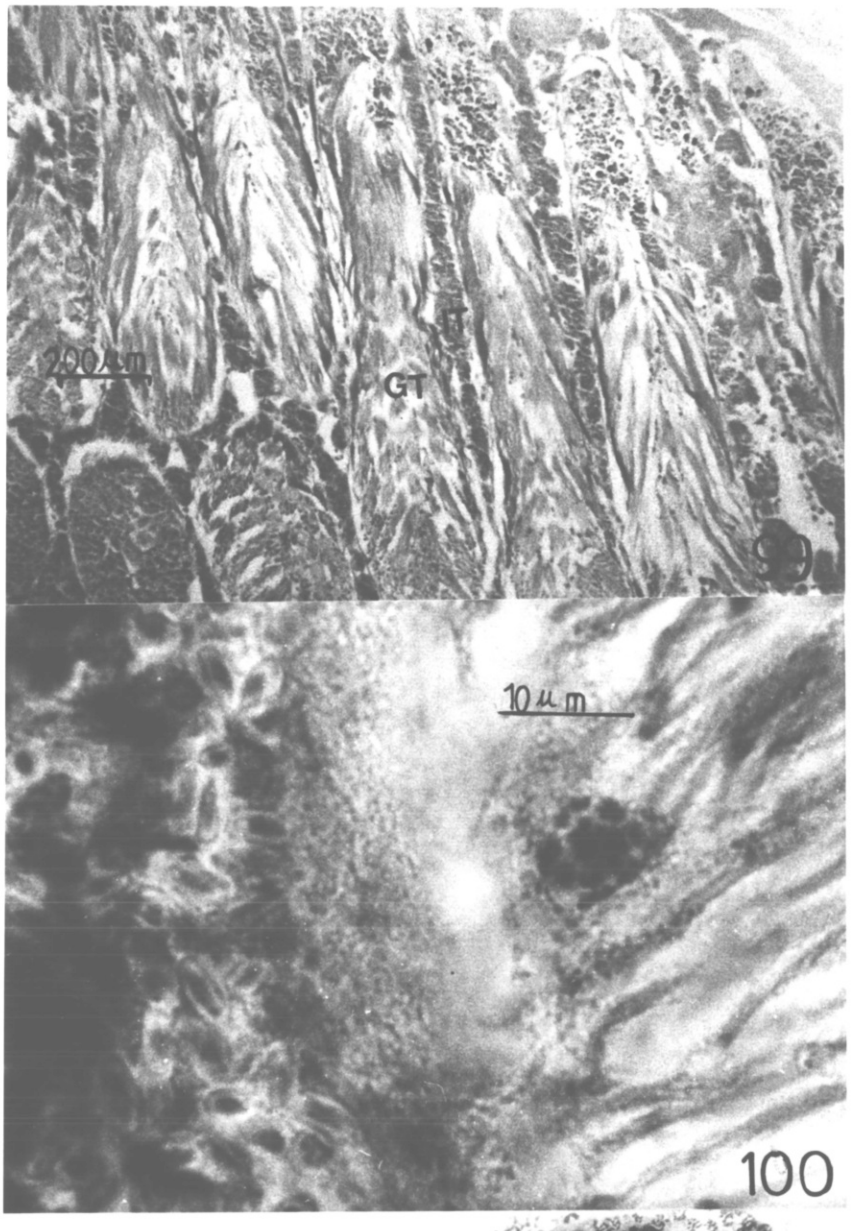

N
N
N
0.5 µm
0.5 µm

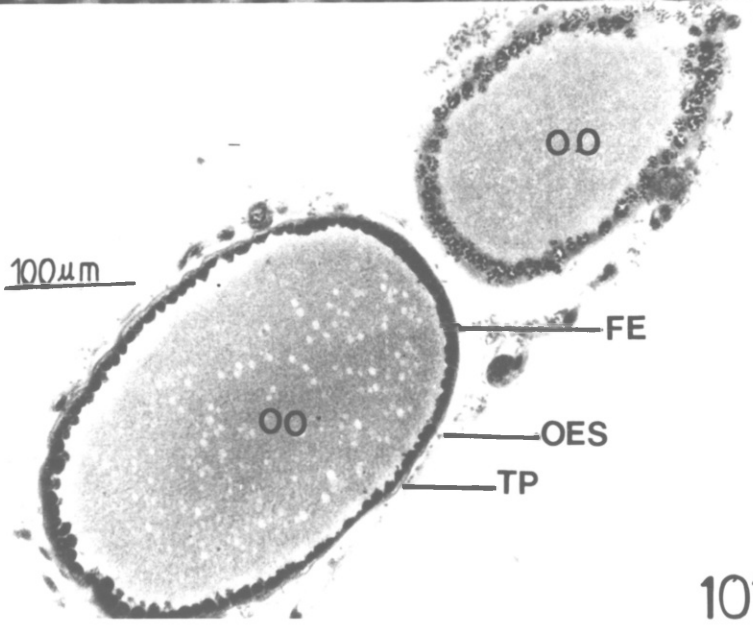
Fig. 99 : Part of a longitudinal section of the testes of Locusta migratoria to show abundant spores in the connective tissue and absence of spores from the tubules

Fig.100 : Testes of L. migratoria showing spores of N. locustae in intertubular tissue adjacent to healthy sperm tubules

Fig.101 : Longitudinal section of oocytes of L. migratoria, enclosed by follicular epithelium, tunica propria and outer epithelial sheath



100

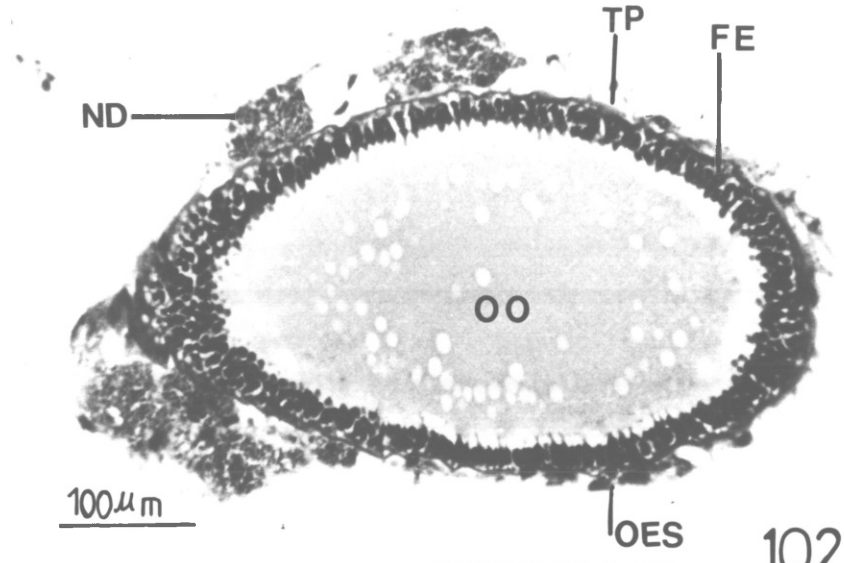


101

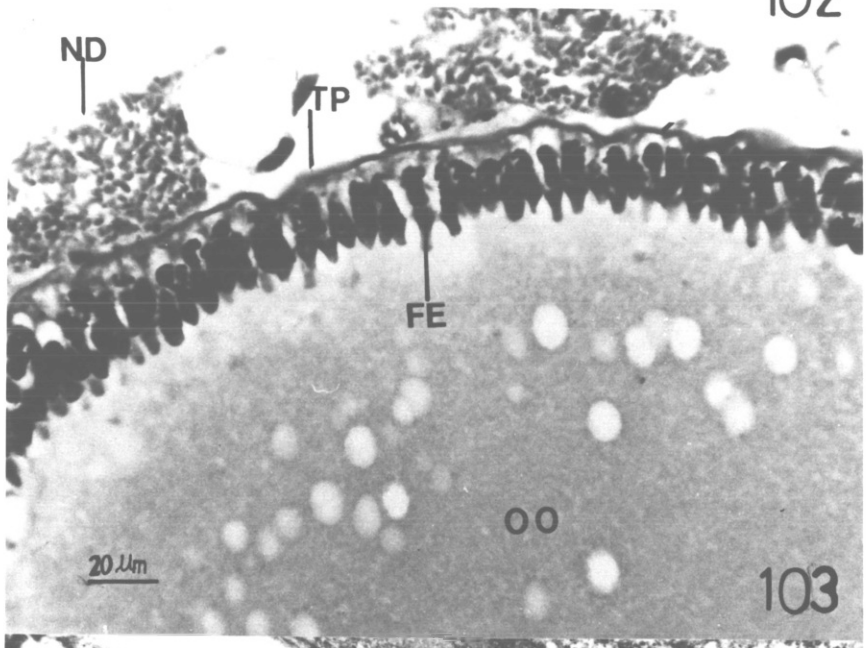
Fig. 102 : Longitudinal section of oocyte of *L. migratoria* : the outer epithelial sheath is infected with *N. locustae* which causes hypertrophy in the form of nodular growth

Fig. 103 : Nodules in the outer epithelial sheath of ovariole of *L. migratoria* filled with spores of *N. locustae*

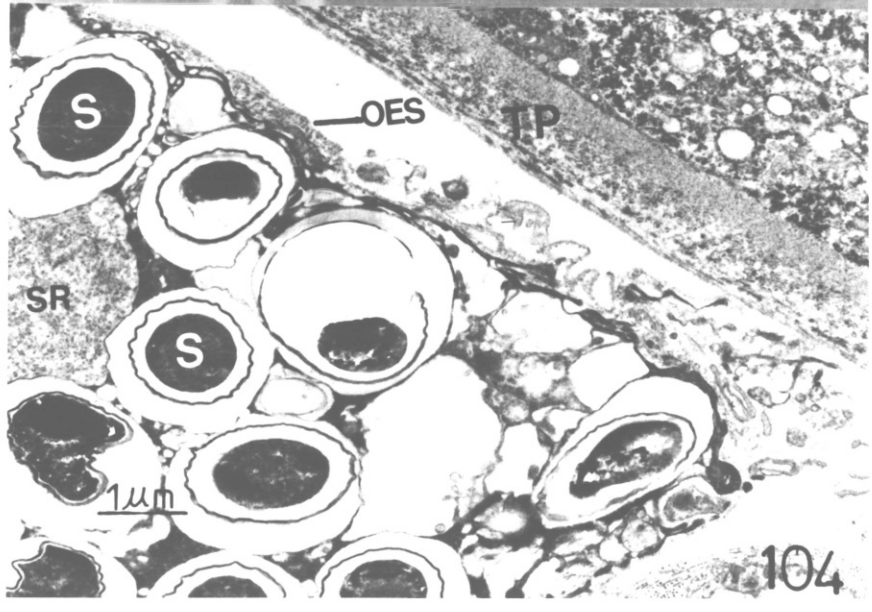
Fig. 104 : Spores and sporogonic stages of *N. locustae* in the outer epithelial sheath of ovariole of *L. migratoria*



102



103



104

Fig. 105 : Meront of N. locustae with simple plasma membrane and 2 nuclei in diplokaryon form.
Arrow points to a vesicle containing electron dense material

Fig. 106 : Meront of N. locustae dividing by binary fission.
Arrow points to a vesicle containing electron dense material

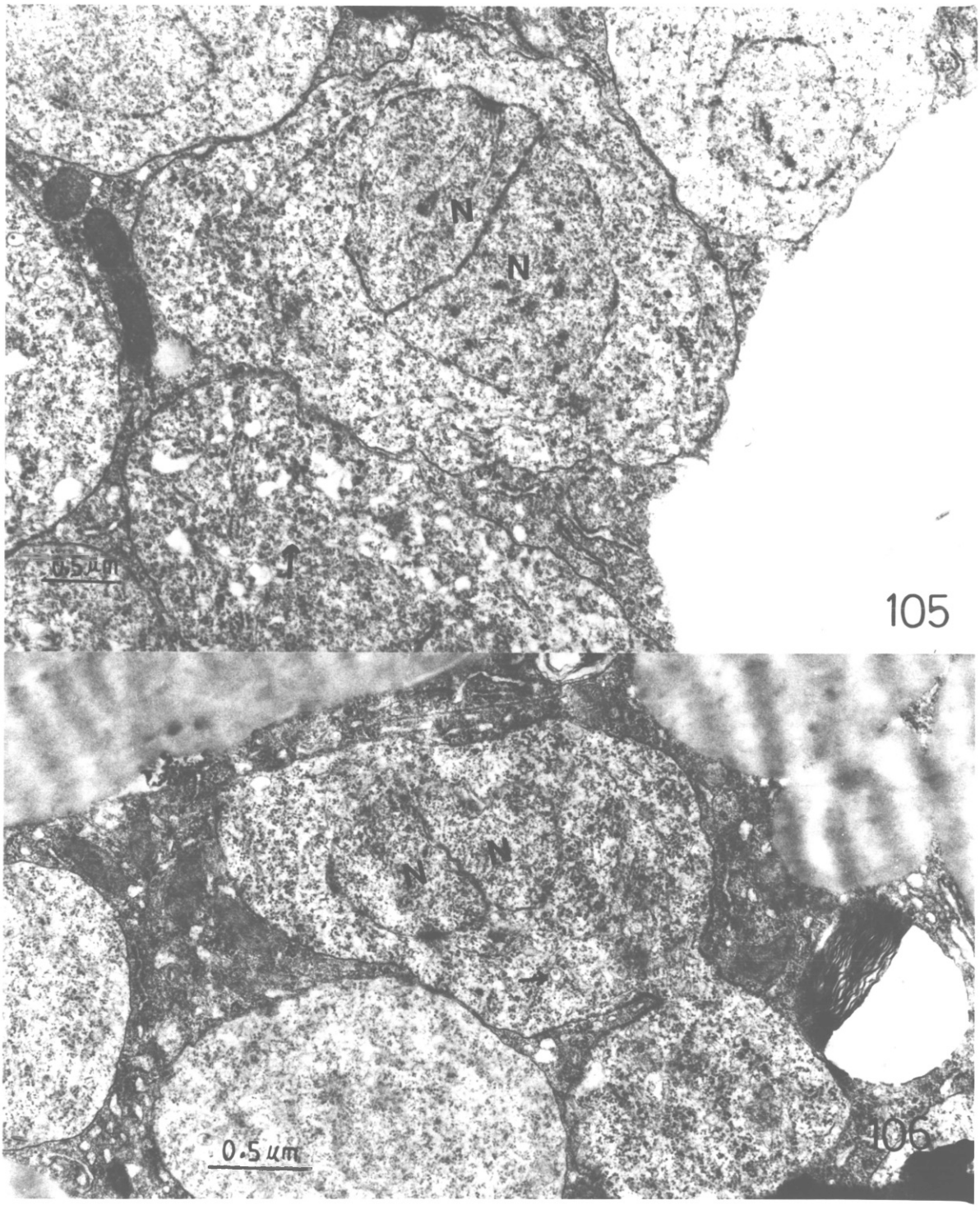
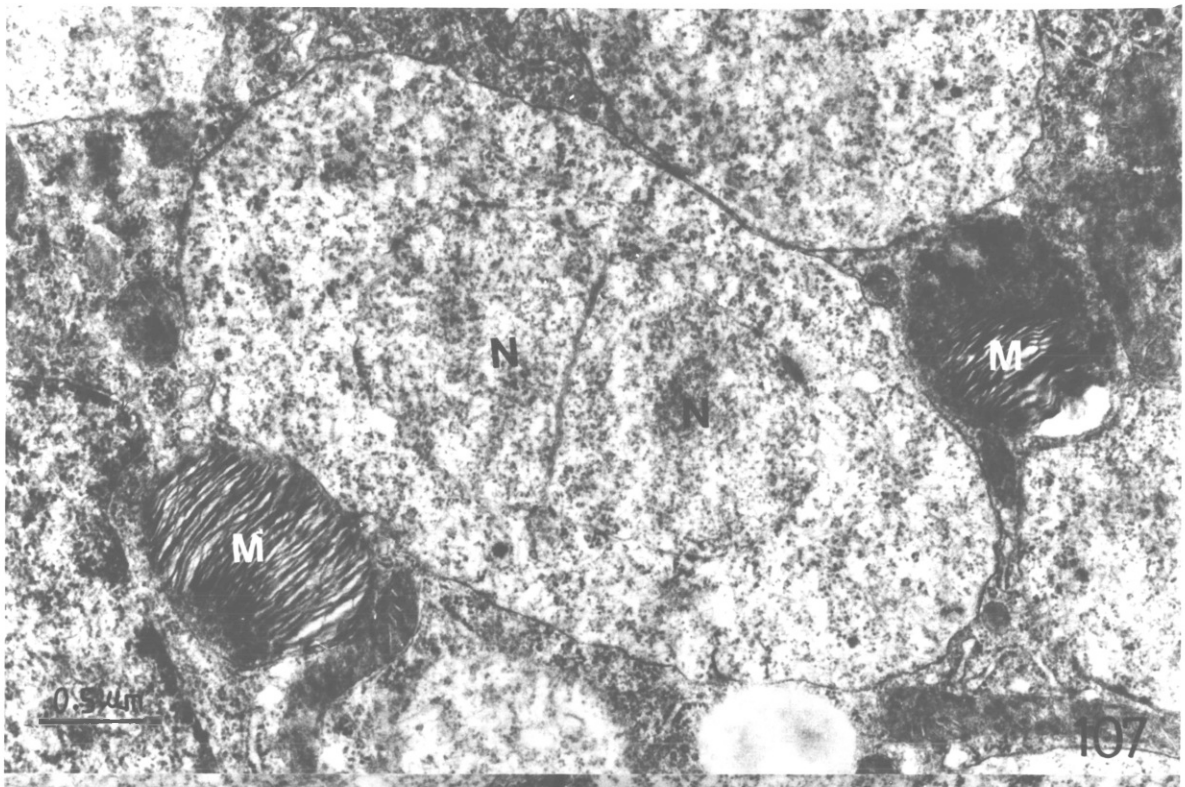
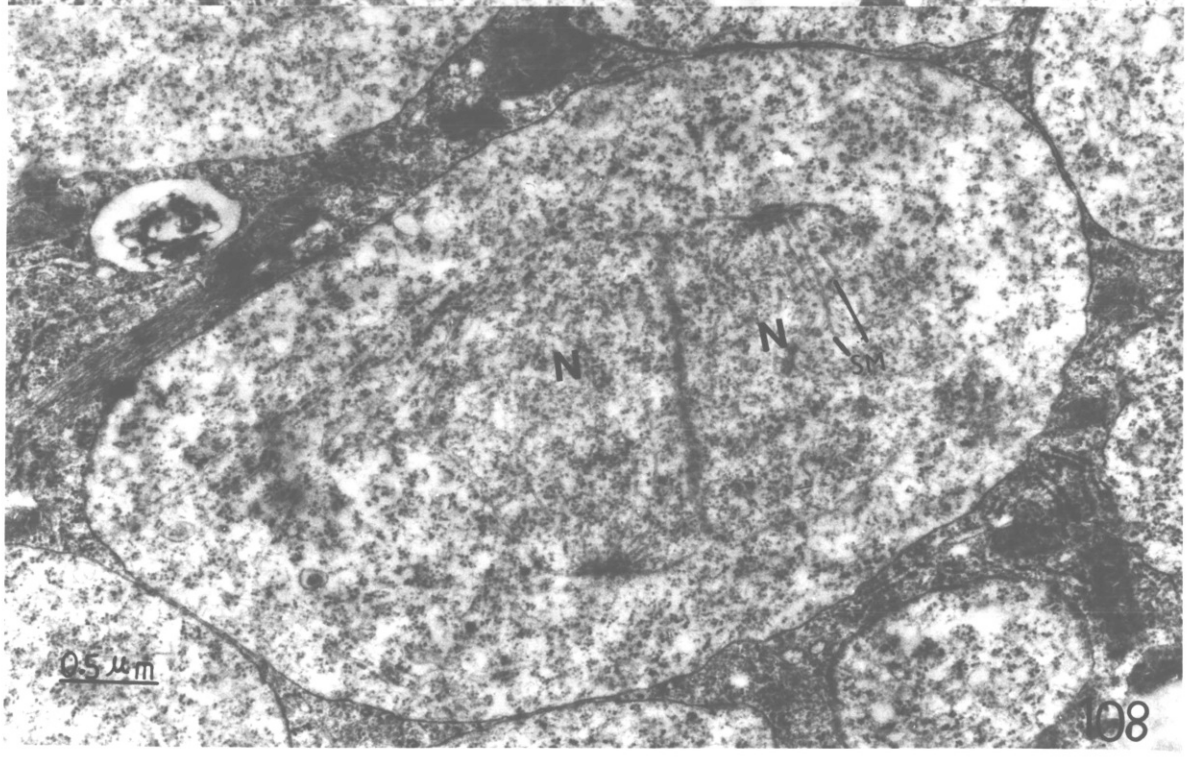

N
N
0.5 µm
105
N
N
0.5 µm
106

Fig. 107 : Mitochondria closely apposed to meronts of N. locustae in the cytoplasm of fat body cells of L. migratoria

Fig. 108 : Spindle plaques formed in the 2 nuclear envelopes of diplokarya of a meront of N. locustae



107

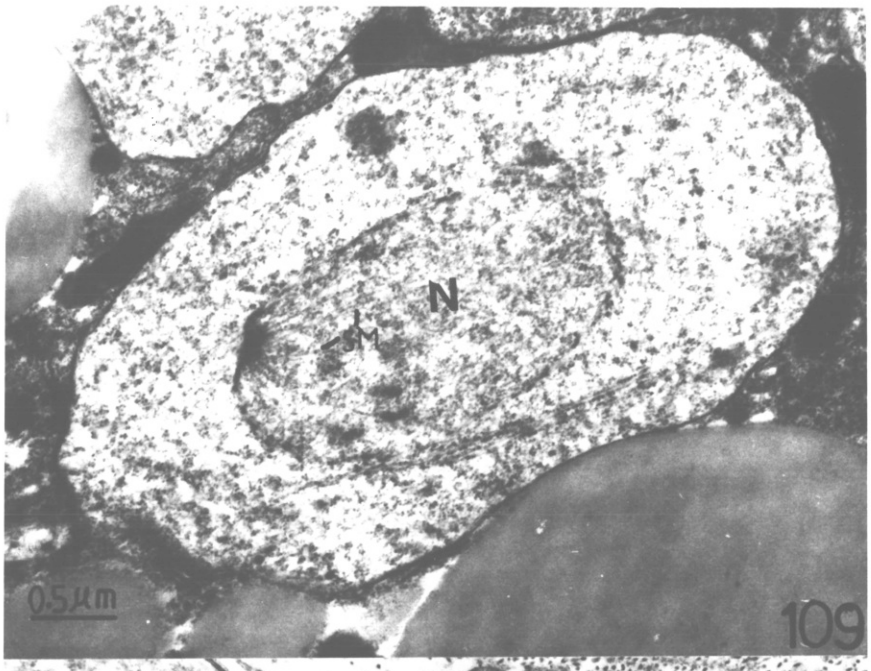


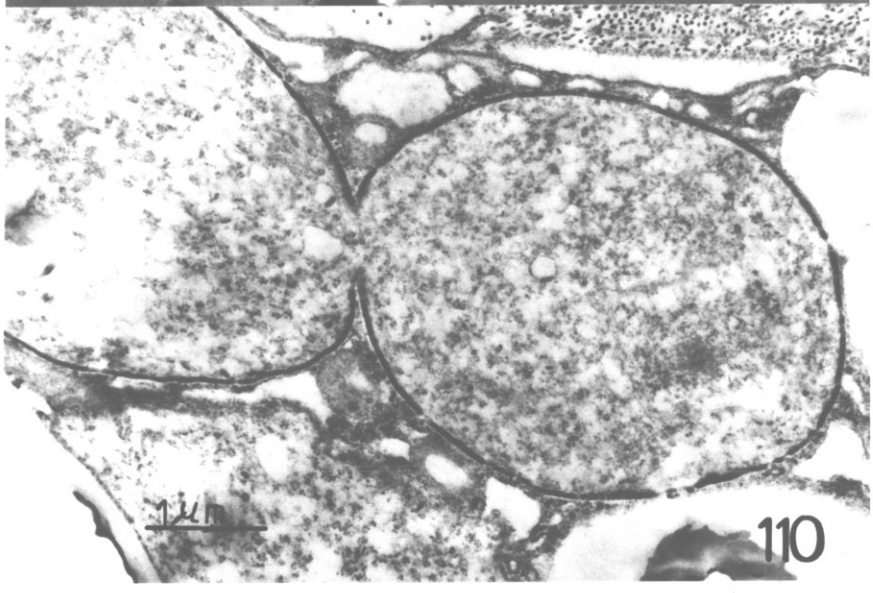
108

Fig. 109 : Meront of N. locustae : spindle plaque with radiating microtubules associated with electron dense material

Fig. 110 : Sporont of N. locustae dividing by binary fission

Fig. 111 : Sporoblast of N. locustae with crenated outlines and disorganized cytoplasm


N
M
0.5µm
109


1µm
110

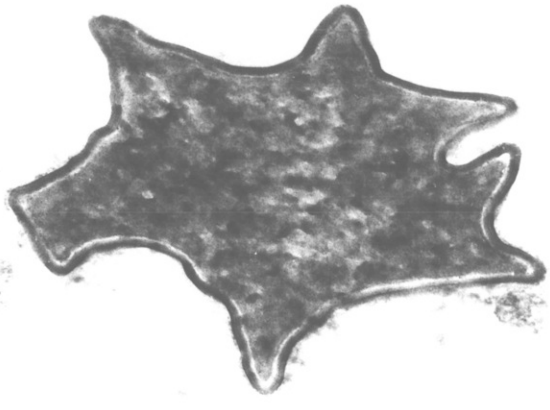
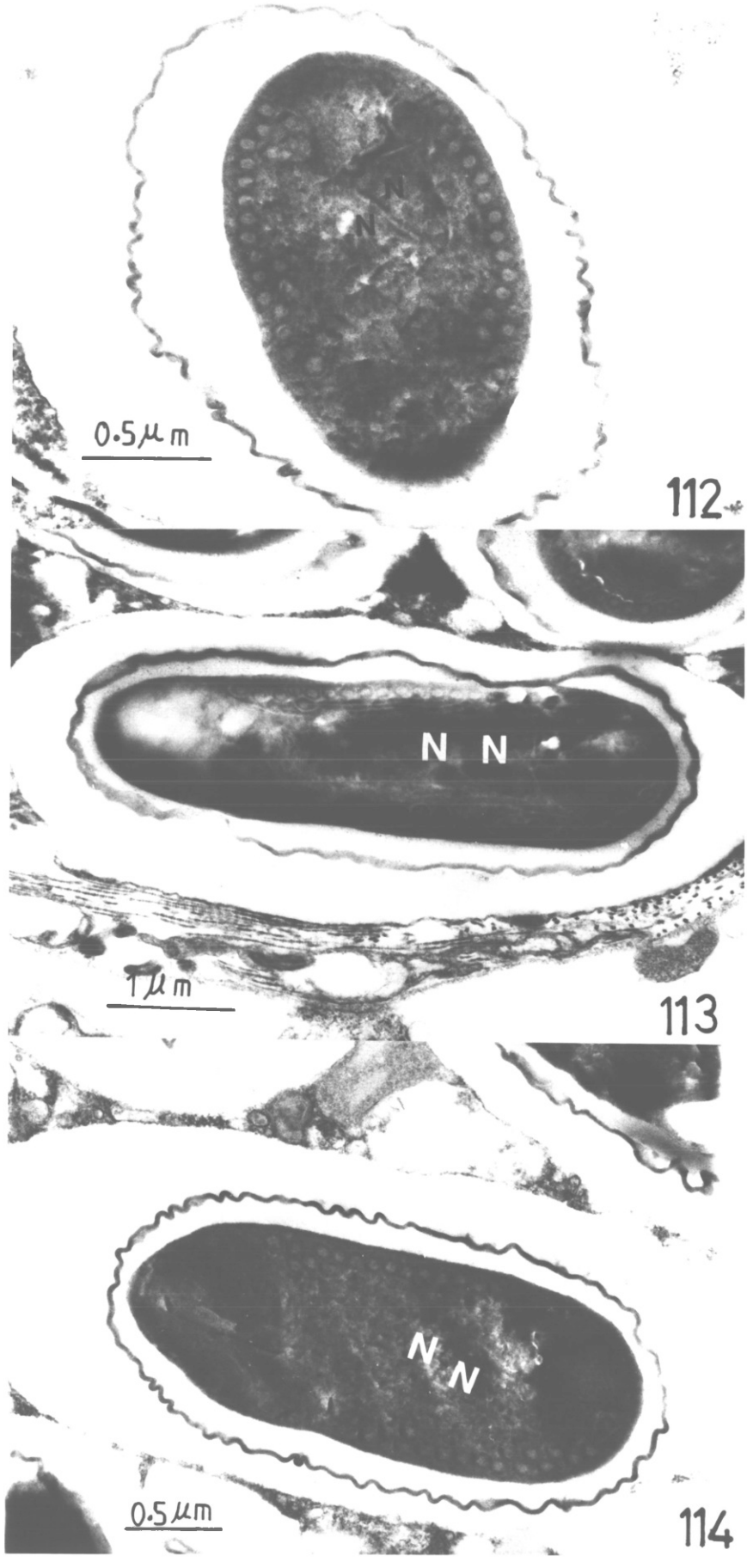

0.5µm
111

Fig. 112 : Developing spore of N. locustae with some overlapping of polar tube coils

Fig. 113 : Spore of N. locustae with 17 - 19 coils and 40^{o} angle of tilt

Fig. 114 : Spore of N. locustae with 2 nuclei in the posterior half and 50^{o} angle of tilt


N
N
0.5 µm
112
N N
1 µm
113
N N
0.5 µm
114

4. DISCUSSION

4.1 Effect of *Nosema heliothidis* on oviposition in *Heliothis zea*

Microsporidia affect insect populations either directly by killing the host or indirectly by reducing the number of oviposited eggs. In this study *Pleistophora operophterae* was so severely pathogenic to an experimental host, *Heliothis zea* that no adults survived to lay eggs even when very small doses of spores (2×10^4 spores/larva) were given. Similarly, there was total mortality before the adult stage was reached in *Oxycatantops praemonatus* and *Catantops sylvestrii* infected with *Nosema locustae*. In the other host/parasite systems investigated in this work there was survival to oviposition. The quantitative effects of the parasite on oviposition were studied in *Heliothis zea* infected with *Nosema heliothidis*.

Thomson (1958a) attributed the reduction in fecundity of *Choristoneura fumiferana* infected with *Perezia fumiferanae* to the inability of female hosts to assimilate their food. Some microsporidia mainly infect the gut epithelium and reduce the functional area of the gut. This was found for *Perezia fumiferanae* in spruce budworm, *Choristoneura fumiferana*, Thomson, 1955; *Nosema sphingidis* in tobacco hornworm, *Manduca sexta*, Brooks, 1970; *Pleistophora* sp. in *Aedes sierrensis*, Sanders and Poinar, 1976; *Plistophora legeri* in *Lobesia botrana*, Lipa 1982; and *Nosema dungeri* in *Isotoma violacea*, Purrini, 1982. Other microsporidia affect

the number of eggs which are oviposited by parasitizing the fat tissue which is the main source of energy for insects. This is especially true for Lepidoptera, which depend on reserves laid down in the fat body by larvae to form their eggs, examples are Vairimorpha plodiae and V. necatrix in Plodia interpunctella, Kellen and Lindegren, 1968; Malone, 1984; V. necatrix in phytophagous Lepidoptera, Maddox, Brooks and Fuxa, 1981; Nosema locustae in Locusta migratoria, Canning, 1953; and Nosema acridophagus in Melanoplus sanguinipes, M. bivittatus and M. differentialis, Henry, 1967.

In many studies the reduction - due to microsporidian infection - of number of eggs oviposited has been quantified. Female larch sawfly Pristiphora erichsonii infected with Thelohania pristiphorae oviposited from 22 to 40 eggs compared with 52 to 59 laid by uninfected females (Smirnoff and Chu, 1968). In orthopteran insects, in which microsporidia invade the fat tissue, the fecundity of adult females is also reduced. The ovaries of Melanoplus gladstoni infected with Nosema locustae rarely contain any eggs (Henry, 1969; 1971b). Also, Melanoplus differentialis did not lay any eggs when infected at third instars with N. locustae at a dose of 10^5 spores/nymph; when infected as fourth instars with the same dose, they produced abnormal egg masses without pods and when infected at fifth instars, they laid normal eggs but fewer than uninfected ones (Henry and Oma, 1981). In lepidotperan insects in which adults depend upon reserved

fat tissue to form eggs and to produce energy, microsporidia significantly reduce the number of oviposited eggs. Thus, Perezia fumiferanae reduced oviposited eggs of Choristoneura fumiferana by 8 - 9% (Thomson, 1958a); Perezia pyraustae reduced oviposition of Pyrausta nubilalis by 47% and even reduced the number of eggs within each egg mass (Zimmack and Brindley, 1957; Kramer, 1959a; Van Denburgh and Burbutis, 1962); Nosema bombycis reduced fecundity of Bombyx mori by 24.7% and 28.9% when treated with 5×10^3 and 5×10^4 spores/larva respectively (Veber and Jasic, 1961); and female Plodia interpunctella, infected with Nosema plodiae and paired with healthy males, laid 21.3% fewer eggs than of healthy ones.

In the case of Heliothis zea, a natural host of Nosema heliothidis, the parasite invades almost all of the larval tissue, especially the midgut epithelium and fat body and spreads to the gonads of adults (Lipa, 1968a; Brooks and Cranford, 1978). Gaugler and Brooks (1975) investigated the sublethal effects of N. heliothidis in H. zea and found that the parasite significantly reduced the fecundity of infected females. Similar results were obtained in the present study, although the infective doses (1×10^6, 3.2×10^6 and 4.4×10^6 spores/larva) were given to third instars instead of 3 days old larvae (1.3×10^6 spores/3 larvae) as given by Gaugler and Brooks. Gaugler and Brooks found 20% reduction in the eggs oviposited by infected females mated with infected males, in the present study the reduction reached to 69%

4.2 Effect of N. heliothidis on males of H. zea

In the present study, when male H. zea, infected with N. heliothidis were paired with uninfected females, the number of eggs oviposited was significantly less than the number laid by uninfected pairs. Kellen and Lindegren (1971) obtained similar results when healthy females of Plodia interpunctella were mated with males infected with Nosema plodiae. These results suggest that infection in males reduces the viability of spermatozoa and that lack of fertilization affects the number of eggs oviposited. This is supported by evidence on oviposition of unfertilized females. Unfertilized females whether infected or not, laid significantly fewer eggs than fertilized females (mean egg lays were 905 $\pm$ 65 eggs for unfertilized uninfected females, 498.6 $\pm$ 30 eggs for unfertilized infected females, 1200.7 $\pm$ 95 eggs for uninfected females mated with uninfected males and 771.4 $\pm$ 82 eggs by infected females mated with uninfected males). In fact, the number of eggs laid by uninfected females caged with infected males (847.9 $\pm$ 73) does not differ significantly from the number laid by unfertilized uninfected females (905 $\pm$ 65) suggesting either that the viability of the spermatozoa was so low that fertilization did not take place or that mating success was affected. Gaugler and Brooks (1975) using the same host and parasite, found that infected males copulate less successfully than uninfected ones and they attributed this to a reduction in vigour. Thomson (1958a) also concluded that microsporidian infection causes a disease characterized by weakness.

4.3 Relationship between spore load of N. heliothidis and fecundity of H. zea

As fecundity depends on the reserve fat tissue, it was expected that the fecundity would correlate with the number of spores harvested. The study illustrated that there was no correlation between fecundity in female H. zea and spore load of N. heliothidis. It has also been found that neither the total number of eggs formed nor the unlaid eggs, correlated with the spore load. However, the viability of eggs laid was greatly affected by the spore load. Thus, the percentage of eggs which hatched, when laid by 8 insects infected transovarially with N. heliothidis and mated with uninfected males, ranged between 82 and 0% (82%, 80%, 70%, 65%, 50%, 25% and 0%) while the percentage of eggs which hatched when laid by 5 uninfected females mated with healthy males ranged between 97.7 and 83.4% (97.7%, 94.1%, 88.7%, 88%, 83.4%). Because the spore load differed from one insect to another, so the reduction in fat tissue in test females had not reached the stationary phase (the state in which all the fat tissue had been used up by the parasite; Weiser, 1978) so there were opportunities for spores to replicate further and for oogonia to undergo oogenesis. As it has been revealed from the present histological studies that the reproductive system is mainly invaded by meronts and sporonts, it follows that the harvest of spores was an indication only of the infected state of the fat tissue. As revealed from this study and from studies which have been

done by Fox and Weiser (1959) using Anopheles gambiae infected with Nosema stegomyiae and Kellen and Lindegren (1968) using Plodia interpunctella infected with Nosema plodiae, oogonia in heavily infected ovaries fail to undergo normal oogenesis. As only a few infected females of H. zea succeeded in laying completely uninfected eggs, it is clear that the fecundity of females depends firstly on the reserved fat tissue and secondly on the degree of infection in the ovarian tissue.

4.4 The difference in spore load of N. heliothidis when H. zea was infected orally and transovarially

The spore load in adult female H. zea, which were infected at 3rd larval instar with a dose of 1×10^6 spores/larva, ranged between 4×10^7 and 1.4×10^8. The spore load of the progeny from these females ranged between 1.4×10^8 and 5.8×10^8. In only one case was the mean spore load of the progeny lower than that of the corresponding mother. Only 2 of the progeny examined, one from each of 2 different mothers, had no infection. Thomson (1958b) reported that progeny of lightly infected females of spruce budworm, Choristoneura fumiferana contained less spores of Perezia fumiferanae than progeny of heavily infected females. He concluded this from dissection of the mothers as adults and their progeny as larvae and by examination of the degree of infection in their tissues. Histological studies in the present work showed that invasion of larval tissues by N. heliothidis was initiated during

embryonic development of H. zea. There was, thus, a greater opportunity for multiplicatin by the microsporidia in the larval tissues of the progeny than in larvae which had been infected at the 3rd instar.

4.5 The relationship between spore load of N. heliothidis in male H. zea and percentage of egg hatch

The present histological studies revealed that spores of microsporidia are capable of invading the testicular cyst cells, which enclose the spermatozoa and nourish them. In heavily infected testes, the testicular cyst cells are disrupted and the spermatozoa are released into the testes, instead of being released in the vasa deferentia, so that many of the spermatozoa are wasted. Kellen and Lindegren (1971) found that when uninfected females of Plodia interpunctella were paired with males infected with Nosema plodiae, there was a poor rate of hatching of the eggs and they attributed this to the damage of the testes caused by the infection. In contrast some authors have found that fertility of eggs is unaffected by infection of male insects with microsporidia. Thus infection with Perezia fumiferanae did not influence the fertility of the eggs of Choristoneura fumiferana (Thomson, 1958a) and infection of Oncopera alboguttata with Pleistophora oncoperae did not significantly affect fertility (Milner and Lutton, 1980). Gaugler and Brooks (1975), using the same parasite and host system as in the present work, reported contrasting results ie. that egg hatch was not significantly affected by infection of male H.

zea with N. heliothidis. As the same time they reported that mating success was influenced by the infection and was reduced by 16.2% and 23% in male insects infected orally and transovarially. They obtained great variations in the data on the percentage of egg hatch of control insects which ranged between 3.7 and 97.9%. These variations might be the explanation of their finding that egg hatches were not affected by infection in male moths, especially as they found, due to the great variation in the number of eggs oviposited, that there were no significant differences in eggs oviposited by healthy females and eggs oviposited by females infected transovarially (at the same time there was significant differences in eggs oviposited by insects infected orally and control insects).

4.6 Effect of N. heliothidis on longevity of adult H. zea

Many references have dealt with the effect of microsporidia on adult longevity and most of them have concluded that there is a significant difference in adult longevity between infected and non infected insects. Thomson (1958a) found that there was a significant difference at the 1% level (t test) in adult longevity of uninfected insects of Choristoneura fumiferana and insects infected with Perezia fumiferanae. There was a highly significant reduction in adult longevity of Pyrausta nubilalis when infected with Perezia pyraustae (Kramer, 1959a). Thomson (1958a) found that, although larval life was extended in infected insects, the reserved food in infected pupae was

less than that of uninfected Choristoneura fumiferana. As this is likely to be true of other microsporidian infections which are located in the fat body, it is to be expected that the longevity of infected adults will be less than that of uninfected adults. Gaugler and Brooks (1975) showed that the mean longevity of adult H. zea infected with N. heliothidis was significantly shorter than that of control adults. In the present study there was only 1 day elongation in larval life for insects treated with dose 1 x 10^6 spores/larva and 2 days for insects treated with 3.2 x 10^6 spores/larva and only 1 day elongation for insects infected transovarially. There was no significant reduction in adult longevity. Gaugler and Brooks (1975) infected 1st instar larvae (with doses 1.3 x 10^6 spore/cup, each cup containing 3 larvae) to study the sublethal effect of infection by N. heliothidis in H. zea. The control insects as well as infected insects (orally and transovarially) entered diapause which gave the parasite more time to multiply in insect tissues. The first aim of the present work was to study generation to generation transmission of microsporidia, so larvae of H. zea were infected at 3rd instar with N. heliothidis with doses 1 x 10^6, 3.2 x 10^6 and 4.4 x 10^6 spores/larva to minimize the number of dead insects due to microsporidian infection and in the present work H. zea did not enter diapause.

4.7 Relationship between dose of Pleistophora operophterae and final spore load in adult H. zea

In the present study it was found that as the dose of P.

operophterae increased the number of spores harvested also increased after the same time interval, as would be expected. Lai and Canning (1983) studied the effect of spore dose of Nosema algerae given at 4th or 5th instar Pieris brassicae, on the spore yield from pupae. With doses of 5 x 10^2, 5 x 10^3 and 5 x 10^4 the corresponding mean spore yields were 1.0 x 10^8, 3.7 x 10^8 and 1.01 x 10^9. Thus, as in the present experiment, higher spore doses gave higher numbers of spores harvested. These results, however, contrast with those of Hostounsky and Weiser (1972), who infected Mamestra brassicae with doses of 5 x 10^3, 5 x 10^4 and 7.2 x 10^5 spores of Vairimorpha plodiae and harvested the spores after 16-18 days. The numbers of spores harvested from the 3 different groups were nearly the same. Henry, Oma, Onsager and Oldacre (1979) got similar results when they infected Melanoplus sanquinipes with doses of 10^4 and 10^5 of Nosema acridophagus and Nosema cuneatum: the spore loads harvested from grasshoppers 20 days later were similar but with a lower dose of 10^2, spore production was lower. Lai and Canning (1983) attributed the results of Henry et al. to the fact that, with the higher doses spores production had reached the stationary phase, in which all the fat body had been used up by the microsporidia and there was no subsequent increase in the number of spores harvested. This was not so with the dose of 10^2 spores. In the present study the spores were also harvested before the infection had reached the stationary phase. The spores harvested ranged between 3 x

10^{10} and 1.4 x 10^{11} for a dose of 2 x 10^{4} and ranged between 4 x 10^{10} and 1.3 x 10^{11} for a dose of 2 x 10^{5}. For a dose of 2 x 10^{6} it ranged between 8 x 10^{6} and 1.6 x 10^{11} spores/insect. The spores probably multiply at the same rate under the same physical conditions, so that an increase in dose would result in and increase in harvested spores.

4.8 Generation to generation transmission

4.8.1 Transmission of microsporidia by male insects

The present study revealed that male H. zea could not transmit N. heliothidis via the contents of the eggs since the smears of surface sterilized eggs of healthy females, paired with diseased males, were devoid of infection. Transovum transmission, i.e. the possibility that spores from infected males could contaminate the surface of eggs, has not been studied. Kellen and Lindegren (1971) showed that 70% of healthy females of Plodia interpunctella harboured spores in the bursa copulatrix after copulation with males infected with N. plodiae but they did not examine whether these spores could contaminate only the surface of the eggs or could enter into the contents of the eggs. Brooks (1968) found stages of N. heliothidis in the gelatinous material in which the bundles of spermatozoa were embedded and he suggested that transmission of microsporidia during copulation was possible. In the present study stages of N. heliothidis were concentrated inside the testes in the testicular cyst cells and intertubular tissues. As they did not infect the developing spermatozoa it is not likely that the spores could be transferred during copulation.

4.8.2 Transmission of microsporidia by female insects

Many studies have revealed that some microsporidia can be transmitted transovarially by their hosts. Most of the microsporidian stages can be involved in the transmission. Smears of eggs of Plodia interpunctella (Kellen and Lindegren, 1973), contained mono- and binucleate trophozoites and spores. Meronts of N. heliothidis were seen in the primary oocytes of a Laphygma exigua (Lipa and Issi, 1979). Sporogonic stages of Orthosoma operophterae and meronts of Nosema wistmansi were observed in non-embryonated eggs of Operophtera brumata (Canning, Wigley and Barker, 1983). Brooks (1968) identified binucleate stages of N. heliothidis in egg smears of H. zea as meronts and moreover found some spores in the smears. The present study has additionally shown that N. heliothidis can be transmitted in the eggs of H. zea as sporonts and sporoblasts. No spores were seen in the smears, but spores which were seen in the ovarial tissue were shown by electron microscopy to be degenerate. It is obvious that the parasite can undergo normal merogony in developing oocytes but is incapable of forming normal spores either in developing oocytes or non embryonated eggs. It is not known what is the mechanism in the oocytes which prevents the parasite from forming healthy mature spores. Kellen and Lindegren (1973) found tailed stages (stages in which the cytoplasm was expanded from one end to form a tail-like extension) of N. plodiae with diplokarya in eggs of Plodia interpunctella and according to the reduction of cytoplasm

and lack of signs of division they considered these stages as arrested or degenerate stages.

Smears of immature eggs (eggs inside the ovaries) infected either with Nosema bombycis or Pleistophora operophterae contained meronts, sporonts, sporoblasts and spores. As all the females had died due to infection, eggs could not be examined after oviposition. As a result it is not known whether meronts and sporonts are involved in the transmission as well as spores. Spores, which were seen in sections of egg follicles were well developed, so in the cases of N. bombycis and P. operophterae, the parasites undergo normal merogony and sporogony in developing eggs of H. zea. Canning, Wigley and Barker (1983) found only free spores and a few small sporophorous vesicles of P. operophterae in smears of non-embryonated eggs of Operophtera brumata. In the present study sections of developing eggs of H. zea revealed abundance of sporophorous vesicles of P. operophterae but free spores were rare. The sporophorous vesicles in the eggs of P. operophterae might be broken up by smearing to give the impression of free spores and few sporophorous vesicles as seen by Canning et al. (1983).

Egg follicles of Laphygma exigua infected with N. heliothidis had a multilayered follicular epithelium instead of the normal single layer of uninfected follicles (Lipa and Issi, 1979). In the present study, egg follicles of H. zea infected with N. bombycis also had a multilayered follicular epithelium and electron microscopy revealed the presence of

parasites in it which must have stimulated the hypertrophy. In contrast the follicular epithelium was uninfected and composed of a single layer in the egg follicles of H. zea when infected with P. operophterae. This was also the case with follicles of H. zea infected with N. heliothidis i.e. the follicular epithelium remained uninfected. This has also been found by Brooks (1968).

In the case of Locusta migratoria, Nosema locustae failed to invade the reproductive tissue except the epithelial sheath of the ovarioles. In the eipthelial sheath the parasite undergoes normal merogony and sporogony. In Lepidoptera and some Diptera, which have polytrophic ovarioles (in which the oocytes alternate with trophocytes) certain microsporidia invade both types of cells and are capable of being transmitted by the transovarial route. In locusts which have panoistic ovaries (in which trophocytes are absent) N. locustae did not invade the oocytes. Although they did not provide cytological evidence, Kellen and Lindegren (1973) suggested that the invasion of egg follicles of Plodia interpunctella by Nosema plodiae starts in the trophocytes, then the infection spreads to the oocytes. Moreover they concluded that the stages developed and multiplied only in the trophocytes and that, after they passed to the oocytes, development was arrested. In the present study all three species examined in H. zea, N. bombycis, P. operophterae and N. heliothidis, developed and multiplied in the oocytes as well as the trophocytes and

underwent merogony and sporogony. In the case of N. heliothidis development was arrested at sporogony and normal spores were not produced. If it is true that infection starts in trophocytes as suggested by Kellen and Lindegren, this could be the reason that follicles of Locusta migratoria which have no trophocytes, did not become infected. However, this point needs further investigation.

Ewen and Mukerji (1980) did not find spores or sporonts of N. locustae in egg pods of Melanoplus sanquinipes, M. packardii and Camnula pellucida nor in the froth which surrounds the egg pods. However, 10% of the progeny at third nymphal instars were infected, but the authors did not show that the infection of the progeny was due to transovum or transovarial transmission. In the present study neither the froth of egg pods from infected females mated with healthy males, nor the froth of pods of healthy females mated with infected males had any sign of infection. Also the progeny of infected parent(s) were free of infection.

4.8.3 Invasion of microsporidia into the embryonic tissue

Nordin (1975) studied the invasion of Nosema sp. to the embryonic tissues of the univoltine insect Malacosoma americanum. Also Canning, Barker, Page and Nicholas (1985) investigated the transmission of Orthosoma operophterae to the embryonic tissues of Operophtera brumata which is a univoltine insect. In both studies, spores were present in the yolk which filled the gut lumen of the embryo. Nordin (1975) found that the infection spread to some tissues of the

larvae but that infections were light. Canning et al. (1985) found that O. operophterae was entirely restricted to the yolk. There were stages in the gut lumen and in residual yolk between embryos and serosa which developed into normal spores. It was assumed that these spores initiated infection in the emerging larvae as the larvae eat their way out of the egg shell. In the present study after differentiation of embryonic tissue within the eggs of H. zea, a multivoltine insect, the sporoblasts and sporonts of N. heliothidis succeed in developing into spores and moderate infections develop in fat tissue and epidermis. Sporonts and sporoblasts, which remained in the yolk outside the embryo, degenerated.

4.9 Ultrastructure of the microsporidia which were used in the study of transovarial transmission

4.9.1 Nosema spp.

Studies on the ultrastructure of the microsporidia were carried out to confirm which stages were involved in transovarial transmission as it is often difficult to distinguish meronts and sporonts at the light microscope level. At the same time the parasites were studied to see whether they developed normally in the female reproductive system. The ultrastructure of one of the species , N. locustae, had only previously been described in the spore stage and as various stages were present in the fat tissues of the hosts investigated in this work, the opportunity was taken to see whether its ultrastructural features were in accord with other species of Nosema.

The study illustrated that N. heliothidis, N. bombycis and N. locustae, in common with other species of Nosema, develop intracellularly in direct contact with the host cell cytoplasm, whether this was in the oocytes and trophocytes (N. heliothidis and N. bombycis) or in fat tissue (N. locustae). Meronts were differentiated from other developmental stages by the limiting membrane. They were surrounded by a simple plasma membrane as is the case for many microsporidia from widely different hosts, e.g. Nosema sp. (= Ameson michaelis) from the crab Callinectes sapidus (Sprague, 1965; Sprague, Vernick and Lloyd, Jr., 1968); Unikaryon legeri, from the trematode, Meigymnophallus minutus, (Canning and Nicholas, 1974); Nosema oryzaephili, from the flour beetle Oryzaephilus surinamensis, (Burges, Canning and Hulls, 1974); and Nosema herpobdellae, from the leech Erpobdella octoculata (Spelling and Young, 1983). There was no sign of an electron dense coat deposited on the plasma membrane as in the case of Nosema whitei (Milner, 1972) or of similar electron dense material interspersed with tubular structures as in the case of Nosema eurytremae (Colley, Joe, Zaman and Canning, 1975) and Nosema algerae (Canning and Sinden, 1973). In the species investigated here, electron dense material was only deposited on the surface at the onset of sporogony. In N. heliothidis and N. bombycis deposition of the dense surface coat commenced at the poles and then spread towards the centre to cover the entire surface. However, in N. locustae, it was deposited

more irregularly on the plasma membrane before forming a complete coat (Fig.110). Sporoblasts were differentiated from spores by the absence of electron lucent endospore.

Meronts were also differentiated from the stages of sporogony by their simple cytoplasmic organization. There were abundant ribosomes and usually a few cisternae of endoplasmic reticulum (er). Er cisternae were not seen in meronts of N. bombycis. Sporonts develop an extensive network of er, which may be related to the active period of synthesis of spore organelles.

The 3 species of Nosema were in accord with diagnosis of the genus, as established by Sprague and Vernick (1971), in that the nuclei were always in diplokaryon arrangement. This confirmed the observation of Ishihara (1970) that the type species of the genus, N. bombycis, has diplokarya.

Meronts of the 3 Nosema spp. divided by simple binary fission, began by invagination of the surface membrane as a groove which became deeper until the opposing membranes fused with each other. Although the division spindles of N. bombycis nuclei were not seen, electron dense intranuclear structures thought to be chromosomes were observed. In N. locustae nuclear division was detected by the presence of centriolar plaques, mictrotubules and chromosomes.

Sprague and Vernick (1971) also included in the diagnosis of the genus Nosema that sporogony is disporous and this was found to be the case in N. bombycis and N. locustae. Division of N. heliothidis sporonts was not seen.

The structure of the spores of N. locustae confirmed the observations of Huger (1960) that there were about 18 turns of the polar tube. In the present study the number of turns ranged from 17 to 19 and the corrugated nature of the exospore, as observed by Huger (1960) was also seen. Huger attributed the corrugation to the method of preparation but this is unlikely because it was seen in only one of the Nosema species examined here and the methods employed were the same for all of them.

Light microscope studies had shown that spores were present in ovarian tissue of H. zea parasitized by N. bombycis. Although other developmental stages were not seen these must have been present because it is unlikely that spores are capable of invading tissues. Further electron microscope studies showed that meronts, sporonts and sporoblasts were present as well as spores. All these stages developed normally in the ovarian tissue, although a few degenerate spores were seen.

In the case of N. heliothidis, light microscopy showed binucleate stages, which could not be identified as meronts or sporonts, and spores in sections of ovaries of H. zea. The invasion of trophocytes by the parasite was not clear except in nearly mature egg follicles, which contained remnants of the trophocytes. Electron microscope studies revealed that the ovarian tissue was invaded by meronts, sporonts and sporoblasts, all of which developed normally in the ovarian tissue. However, electron microscopy revealed

that the spores were degenerate and could not be responsible for transovarial transmission.

A feature of the meronts of N. heliothidis, not seen in the other Nosema spp. were 26nm diameter tubular structures which extended from the surface of the parasite into spaces digested in the host cell cytoplasm. At first they arranged in parallel bundles then they became narrower (20nm) and randomly distributed in the degenerating cytoplasm. Similar tubular structures were seen by Canning and Sinden (1973) in Nosema algerae in close contact with the surface of meronts and sometimes free in the disrupted cytoplasm of the host, Anopheles stephensi . Also tubular structures, 50nm in diameter were embedded in osmiophilic material covering the plasma membrane of Nosema eurytremae in Echinostoma malayanum (Colley, Joe, Zaman and Canning, 1975). Canning and Sinden (1973) suggested that the tubular structures are secreted by the parasite. The present study supports this suggestion because if the tubular structures were secreted by the host, similar structures should appear in association with N. bombycis in the same host. The exact function of the tubular structures is not known but they may be used to aid the digestion of the cell cytoplasm or convey the soluble products to the parasite membrane. It was interesting to note that spores both of N. heliothidis and N. bombycis (which does not develop tubular structures) which develop in direct contact with the host cell cytoplasm without forming a space around them were degenerate.

4.9.2 *Pleistophora operophterae*

The ultrastructure of *P. operophterae* was studied in the ovarial tissue of *H. zea*. The developmental stages were seen within vesicles, separated from the host cytoplasm, by a membrane of unknown origin. All developmental stages and spores within these vesicles were seen in the trophocytes and oocytes.

The same parasite was studied in gut cells and silk glands of *Operophtera brumata* (Canning, Barker, Nicholas and Page, In press). Canning *et al*. transferred *P. operophterae* to a new genus *Cystosporogenes*, in which all stages develop in vesicles separated from host cytoplasm by a membrane-like envelope. Meronts lie separately in vesicles and have simple plasma membrane. Sporonts divide by budding to form sporoblasts. Stages in ovarial tissue, as seen in the present study, do not differ ultrastructurally from the stages seen by Canning *et al*. in other tissues.

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APPENDIX 1

Semi-synthetic diet for rearing *Heliothis zea*

(Modification of Hoffman's Tobacco Hornworm diet; Hunter, Crook and Entwhistle, 1984).

1. Mix together in a blender

Agar	15 g
Casein	26.4 g
Wheat germ	57.6 g
Wesson's salts*	7.5 g
Yeast powder	11.4 g
Sucrose	23.4 g
Cholesterol	0.75g
Methyl para-hydroxy-benzoate	0.75g
Sorbic acid	1.2 g

2. Add and mix thoroughly:

Water	690 ml
4M KoH	3.75ml
Linseed oil	1.5 ml

3. Autoclave mixture in a covered vessel for 20 min at 20 lb.per sq.in.

   Cool to 60 - 70^{o}C

4. Add and mix thoroughly:

Vitamin/antibiotic mix**	4.5 g
Choline chloride	0.75g

5. Dispense into No.10 Cristaseal polypots to a depth of about 1 cm and store in refrigerator for up to 3-4 weeks.

Appendix 1 (contd)

* Wesson's salts

$CaCo_3$	120 g
$K_2 HPO_4$	129 g
$CaHPO_4 .2H_2O$	30 g
$MgSO_4 .7H_2O$	40.8 g
NaCl	67 g
$FeC_6H_5O_2 .5H_2O$	11 g
KI	0.32g
$MnSO_4 .4H_2O$	2 g
$ZnCl_2$	0.1 g
$CuSo_4 .5H_2O$	0.12g

** Vitamin/antibiotic mix

Nicotinic acid	5 g
Clacium pantothenate	5 g
Riboflavin	2.5 g
Aneurine hydrochloride	1.25g
Pyridoxine hydrochloride	1.25g
Folic acid	1.25g
Biotin	0.1 g
Cyanocobalamine	0.01g

For every gram of this mixture, add

Streptomycin sulphate	2 g
Aureomycin (veterinary soluble powder, 25g/lb)	18 g
Ascorbic acid	40 g

Mix well and store in refrigerator.

APPENDIX 2

Preparation of specimens for light microscopy

Fixation

Carnoy's fluid:

Absolute alcohol	60 ml
Chloroform	30 ml
Acetic acid	10 ml

Fix tissue for about 45 min according to size, then transfer to 90% alcohol

Dehydration

1.90% alcohol	15 min
2.90% alcohol	15 min
3.Celloidin 1% solution in methyl benzoate	1 h

Run solution to the bottom of alcohol and let the specimen sink before removing alcohol

4.Fresh colloidin 1% solution in methyl benzoate	overnight
5.Further fresh colloidin 1% solution in methyl benzoate	2 h
6.Benzene	15 min
7.Benzene at 30°C saturated with paraffin wax (M.P.= 58°C)	30 min

Embedded in paraffin wax (M.P. = 58°C)

Sections (5-6 μm)

Slides placed for 5 min in 60°C oven

Staining

1.Xylene (two changes)	2 min

Appendix 2 (contd)

2.95% alcohol	2 min
3.90% alcohol	2 min
4.80% alcohol	2 min
5.70% alcohol	2 min
6.60% alcohol	2 min
7.50% alcohol	2 min
8.40% alcohol	2 min
9.Giemsa stain	5 min
10.Differentiate with 15% Colophonium in acetone for	2 secs
11.Wash in acetone	
12.Wash in mixture of 70% acetone + 30% cylene	
13.Clean in xylene (two changes)	15 min
14.Mount in green euparal	

Giemsa stain

100 ml Buffer

10 ml Giemsa stain

10 ml Acetone

10 ml Methanol

Buffer solution, pH.7.2

Potassium dihydrogen orthophosphate	2.1g
Di-sodium Hydrogen orthophosphate	5 g

Dissolve in 1000 ml distilled water

Used for thin sections as 1 part buffer + 9 parts tap water

Appendix 2 (contd)

Alcoholic Bouin's fluid

40% formalin	30 ml
Saturated picric acid	12.5ml
Glacial acetic acid	7.5ml
Absolute ethanol	48 ml
Distilled water	15 ml

APPENDIX 3

Gram alum/Heidenhain's stain

Gram alum (Heidenhain's No.1)	overnight
Rinse in tap water	
Heidenhain's stain (No.2)	overnight
Rince in tap water	
Differentiate in half strength Gram alum (40 ml No.1 + 40 ml distilled water)	
Wash 30 min in running tap water	
Dehydration	
1.30% alcohol	2 min
2.50% alcohol	2 min
3.70% alcohol	2 min
4.90% alcohol	2 min
5.100% alcohol	2 min
6.Xylene	2 min
7.Mounted in Canada balsam	

APPENDIX 4

Preparation of specimens for transmission electron microscopy

Karnovsky's fluid

Mix	Paraformaldehyde	2 g
	$Cacl_2$	25 mg
	Distilled water	20 ml

Heat to 65°C, add 2 drops 1 M NaoH;
Stir until clear, Cool to room temperature,
Add 10 ml of 25% glutaraldehyde and make up
to 50 ml with 0.2 M. Sodium cacodylate buffer,
pH.7.4

1. Karnovsky's fluid for 10 min at room temperature
2. Karnovsky's fluid for 1 h at 4°C
3. 0.12 M Sodium cacodylate (two changes) for 15 min at 4°C
4. 2.5% (W/V) OSO_4 in 0.1 M Sodium cacodylate, 1 h, at 4°C, on rotator
5. 0.1 M Sodium acetate (two changes), for 10 min at 4°C, on rotator
6. 0.25% (W/V) Uranyl acetate in water for 1 h at 4°C
7. 0.1 M Sodium acetate (two changes) for 10 min at 4°C, on rotator
8. 35% acetone for 5 min at 4°C
9. 50% acetone for 5 min at 4°C
10. 70% acetone, with 1% Uranyl acetate and 1% phospho-tungstic acid, overnight at 4°C
11. 90% acetone for 10 min at room temperature

Appendix 4 (contd)

12. 100% acetone (three changes) for 15 min at room temperature
13. 50/50 acetone/Spurr's resin, overnight, at room temperature on rotator
14. Spurr's resin, all day at room temperature on rotator
15. Embed in fresh Spurr's resin and polymerize in 60°C oven overnight

Post-staining of sections

Reynold's lead citrate

Lead citrate	0.28g
Sodium citrate	1.76g

Dissolve separately in 15 ml boiled distilled water, then mix together in a 50 ml volumetric flask

Add 8 ml of 1 M NaoH, and dilute total to 50 ml.

1. Float grids, section-side downwards, on drops of 2% aqueous uranyl acetate on dental wax for 15 min
2. Pick up grids and wash with clean distilled water
3. Dry grids on filter paper
4. Place grids, section-side downwards, on drops of Reynold's lead citrate on dental wax in a closed petri dish with some pellets of NaoH. Leave 10 min.
5. Pick up grids and wash with 0.02 M NaoH
6. Wash grids by dipping repeatedly in two beakers of boiled distilled water, blot dry and examine.

APPENDIX 5

Number of eggs laid by adult H. zea treated with N. heliothidis with dose 1 x 10^6 spores/larva at 3rd larval instar

Uninfected ♀ x Uninfected ♂	Infected ♀ x Infected ♂	Infected ♀ x Uninfected ♂	Uninfected ♀ x Infected ♂
790	362	2	195
410	390	427	405
430	305	90	415
815	125	205	90
1160	180	168	190
840	50	75	645
660	-	0	540
720	-	-	170

Number of eggs laid by adult H. zea treated with N. heliothidis at 3rd larval stage with dose 3.2 x 10^6 spores/larva

Uninfected ♂ x Uninfected ♀	Infected ♂ x Infected ♀	Uninfected ♂ x Infected ♀	Infected ♂ x Uninfected ♀
1004	0	565	225
581	85	20	226
420	185	265	75
791	100	338	648
1000	542	290	195
890	0	250	-
670	10	10	-
-	105	460	-
-	0	150	-

Appendix 5 (contd)

Number of eggs laid by adult H. zea treated with N. heliothidis at 3rd larval instar with dose 4.4×10^6 spore/larva

Uninfected ♂ x Uninfected ♀	Infected ♂ x Infected ♀	Uninfected ♂ x Infected ♀	Infected ♂ x Uninfected ♀
990	420	235	610
591	360	409	220
1135	60	225	360
420	270	450	750
910	680	25	270
300	0	10	453
925	440	160	160
830	-	60	1130
1333	-	140	214
416	-	780	730

APPENDIX 6

Life cycle and adult longevity as % of total life cycle of uninfected H. zea and H. zea infected with N. heliothidis at 3rd instar with does 1 x 10^6 spores/larva

Uninfected insect			Infected insect		
Total life cycle	Adult longevity	Adult longevity as % of total life cycle	Total life cycle	Adult longevity	Adult longevity as % of total life cycle
38	6	15.7895	44	12	27.2727
44	11	25.0000	38	6	15.7895
44	13	29.5455	39	6	15.3846
44	12	27.2727	42	8	19.0475
39	8	20.5128	41	8	19.5122
40	8	20.0000	41	9	21.9512
42	10	23.8095	42	10	23.8095
42	9	21.4286	39	6	15.3846
39	8	20.5128	43	10	23.2558
40	9	22.5000	42	10	23.9095
41	9	21.9512	39	8	20.5128
40	9	22.5000	35	2	5.7143
40	9	22.5000	41	9	21.9512
40	8	20.0000	37	4	10.8108
44	11	25.0000	40	8	20.0000
42	10	23.8095	40	9	22.5000
39	8	20.5128	43	11	25.5814
41	9	21.9512	41	8	19.5122
42	10	23.8095	44	11	25.0000
			40	7	17.5000
			38	6	15.7895
			42	9	21.4886
			41	8	19.5122
			44	11	25.0000
			40	7	17.5000
			36	3	8.3333
			40	7	17.5000

Appendix 6 contd.

Life cycle and adult longevity as % of total life cycle of uninfected H. zea and H. zea infected with N. heliothidis at 3rd larval instar with dose 3.2 x 10^6 spores/larva

Uninfected insect			Infected insect		
Total life cycle	Adult longevity as % of total life cycle	Adult longevity	Total life cycle	Adult longevity as % of total life cycle	Adult longevity
44	12	27.2727	45	10	22.2222
50	13	26.000	48	13	27.0833
41	7	17.0732	40	5	12.5000
41	6	14.6341	42	9	21.4286
46	14	30.4384	46	13	28.2609
41	9	21.9512	42	9	21.4286
46	14	30.4348	44	10	22.7273
44	10	22.7273	50	13	26.0000
45	13	28.8889	43	7	16.2791
43	10	23.2558	42	7	16.6667
50	17	34.0000	46	10	21.7391
43	10	23.2558	48	13	27.0833
44	12	27.2727	47	14	29.7872
51	18	35.2941	43	10	23.2558
46	14	30.4348	45	12	26.6667
41	9	21.9512	42	9	21.4286
42	9	21.4286	43	10	23.2558
47	14	29.7878	45	11	23.9130
44	12	27.2727	45	10	22.2222
42	11	26.1905	43	10	23.2558
45	13	28.8889	41	8	19.5122
44	15	34.0909	47	11	23.4043
42	12	28.5714	46	9	19.5652
50	18	36.0000	42	4	9.5238
41	8	19.5122	41	2	4.8780
48	18	37.5000	45	3	6.6667
43	14	32.5581	49	7	14.2857
42	10	23.8095	52	10	19.2308
40	8	20.0000	48	10	20.8333
42	12	28.5714	42	10	23.2558

Appendix 6 contd.

Life cycle, and adult longevity as % of total life cycle of uninfected progeny of H. zea and progeny infected trans-ovarially with N. heliothidis

Uninfected insect			Infected insect		
Total life cycle	Adult longevity	Adult longevity as % of total life cycle	Total life cycle	Adult longevity	Adult longevity as % of total life cycle
52	20	38.4615	41	9	21.9512
45	17	37.7778	47	16	34.0426
52	23	44.2308	46	13	28.2609
49	21	42.8571	49	18	36.7347
43	13	30.2326	50	17	34.0000
39	11	28.2051	38	5	13.1579
52	20	38.4615	46	13	28.2609
47	16	34.0426	43	11	25.5814
43	12	27.9070	47	13	27.6596
47	16	34.0426	47	15	31.9149
53	20	37.7358	44	12	27.2727
46	15	32.6087	41	8	19.5122
48	15	31.2500	51	17	33.3333
40	8	20.0000	47	16	34.0426
40	5	12.5000	47	15	31.9149
50	18	36.0000	48	16	33.3333
44	11	25.0000	46	13	28.2609
45	10	22.2222	48	17	35.4167
46	15	32.6087	37	4	10.8108
46	15	32.6087	48	13	27.0833
43	11	25.5814	49	15	30.6122
43	12	27.9070	48	14	29.1667
46	15	32.6087	50	15	30.0000
52	18	34.6154	49	15	30.6122
41	8	19.5122	37	3	8.1081

.../contd

Appendix 6 contd.

Unifected insect			Infected insect		
Total life cycle	Adult longevity	Adult longevity as % of total life cycle	Total life cycle	Adult longevity	Adult longevity as % of total life cycle
46	15	32.6087	40	6	15.0000
48	19	39.5833	46	14	30.4348
49	14	28.5714	44	11	25.0000
48	16	33.3333	45	13	28.8889
55	24	43.6364	45	15	33.3333
51	17	33.3333	47	14	29.7872
			49	17	34.6939
			44	13	29.5455
			51	18	35.2941
			47	16	34.0426

Appendix 7.

Spore loads of N. heliothidis and fecundity of individual female H. zea, infected at 3rd larval instar with doses of 1×10^6 spores/larva

No of spore load/ female	Total no. of eggs laid / female
6×10^7	390
6×10^7	168
8×10^7	75
9×10^7	125
9×10^7	50
9×10^7	362
1×10^8	305
1×10^8	180
1×10^8	427
1×10^8	90
1×10^8	205
2×10^8	2

Appendix 8.

Spore load of *N. heliothidis* and fecundity of individual female *H. zea*, which has been infected transovarially

No of spore load/ female	Total no.of eggs laid/female
3×10^7	395
4×10^7	260
6×10^7	360
6×10^7	168
8×10^7	75
9×10^7	380
9×10^7	362
9×10^7	150
9×10^7	125
9×10^7	50
1×10^8	427
1×10^8	305
1×10^8	285
1×10^8	205
1×10^8	180
1×10^8	90
1.5×10^8	95
2×10^8	970
2×10^8	740
2×10^8	25
2×10^8	2
4×10^8	710
4×10^8	240

APPENDIX 9

Spore load of N. heliothidis in adult H. zea infected orally at 3rd larval instar with dose $1x10^6$ spores/ larva and adult H. zea infected transovarially

Adult infected orally / Progeny infected transovarially	4×10^7	6×10^7	6×10^7	$7.5x10^7$	9×10^7	1×10^8	$1.1x10^8$	$1.2x10^8$	$1.2x10^8$	$1.3x10^8$	$1.3x10^8$	$1.4x10^8$
1	$2.8x10^8$	2×10^8	$1.4x10^8$	$1.8x10^8$	$1.4x10^8$	$2.7x10^8$	0	0	$1.6x10^8$	$3.5x10^7$	$2.1x10^8$	$2.3x10^8$
2	$4.2x10^8$	3×10^8	$1.5x10^8$	$2.1x10^8$	$2.1x10^8$	$2.8x10^8$	3×10^7	$1.7x10^8$	$1.9x10^8$	$3.5x10^7$	$2.5x10^8$	$2.6x10^8$
3	$3.9x10^8$	$3.3x10^8$	$2.4x10^8$	$2.3x10^8$	$2.2x10^8$	$3.8x10^8$	2×10^8	$1.8x10^8$	$2.6x10^8$	6×10^7	$4.3x10^8$	$2.7x10^8$
4	$2.6x10^8$	4×10^8	$2.7x10^8$	$2.3x10^8$	$2.3x10^8$		$3.1x10^8$	$1.8x10^8$	$2.7x10^8$	$6.5x10^7$		3×10^8
5			$3.6x10^8$		$2.5x10^8$			$1.9x10^8$	$3.3x10^8$	$9.7x10^7$		$3.7x10^8$
6					$2.7x10^8$			$2.3x10^8$	$3.3x10^8$	$4.2x10^7$		$5.8x10^8$
7					$2.7x10^8$			$3.6x10^8$				
8					$3.5x10^8$							

Appendix 10.

Spore load of male and female H. zea infected transovarially with N. heliothidis and % of egg hatch

Spore load/ male	Spore load/ female	% of egg hatch
6×10^7	3×10^7	75
2×10^8	3.6×10^8	0
2.4×10^8	1×10^8	68
2.7×10^8	2.3×10^8	93
2.7×10^8	4.2×10^8	61
2.75×10^8	4.2×10^8	50
2.8×10^8	2.6×10^8	50
2.9×10^8	1.7×10^8	55
2.9×10^8	3.6×10^8	0
3.0×10^8	2.7×10^8	25
3.1×10^8	9.3 x 107	55
3.2×10^8	9×10^7	32
4.2×10^8	1.8×10^8	0
5.8×10^8	4×10^8	0