AN ANALYSIS OF THE CELLULAR DEFENCE REACTION IN BARK-BORING BEETLES INFECTED BY THE HAEMOCOELIC NEMATODE *CONTORTYLENCHUS* SP.

by

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ABSTRACT

The bark-boring beetle Ips sexdentatus (Boern) were collected in large numbers from felled pine trees in Southern England throughout the year. The incidence of parasitism by Contortylenchus diplogaster and encapsulation of the nematode have been studied. The encapsulated nematodes are retained in the haemocoel of only the pupal and adult stage, without any adverse effect on the parasite's development. All nematodes in the pupal stage were encapsulated, but many had escaped from their capsules and were free in the haemolymph of adult beetles. The extent to which nematodes remained encapsulated in the adult beetle decreased with increasing parasite burden.

The morphometrics of Contortylenchus were studied and as a result this species was synonymized with C. diplogaster Ruhm (1956). The variations were considered not a valid criterion for species differentiation.

The effect of parasitism on the parasite's volume and fecundity together with the effect of the parasite's density on the development and fecundity of the host were reported and discussed.

The osmotic relationships of C. diplogaster have been investigated. C. dirlogaster lives in an environment which is hypertonic to the body fluid. Basically, the process

of osmotic regulation is similar to the process in other animal parasitic nematodes. The ability of osmoregulation, however is more active in hypertonic than in hypotic media.

A quantitative and qualitative study was made of the haemocytes of I. sexdentatus together with the effect of parasitism on the total and differential haemocytes count.

C. diplogaster exposed to a cellular immune reaction in the host haemolymph resulted in the formation of haemocytic capsule. The haemocytes involved in capsule formation were polymorphic plasmatocytes, amoebocytes and granular haemocytes. These cells, which aggregate around the invaded nematode, then undergo a complex series of structural modifications, apparently synchronized with the development of the nematode. The capsule enlarges and becomes a cellular, multinucleate, hollow sphere, composed of approximately 250 nuclei, which enlarge and become amoeboid in shape; they decline and eventually the capsule becomes non-nucleate. Before pupation was completed minute tracheoles were found to ramify through these haemocytes. Tracheation was stimulated by the presence of the nematode. Eighty percent of the capsules found in adult beetles were tracheate.

Throughout its development the relationships between

the volume of the capsule and the capsule nuclei were investigated.

The fine structure of the capsule throughout its development and the haemocytes which form there have been studied by electron microscopy.

Ultrastructure of the body wall of young and old parasitic female C. diplogaster has been studied. This nematode lack a normal cuticle. A membranous layer covering the body was found. The formation of this layer has been discussed in relation to nutrition.

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1. INTRODUCTION

1.1. Ecological and Biological Aspects of the Host - Parasite Relationship

Bark beetles (Coleoptera, Scolytidae), are known to be serious forest pests in United States and Europe. They bore their galleries through the bark on its inner surface (Anderson, 1960). Ips species attack the dead trees and are considered as secondary pests of their host.

Ips sexdentatus, Boern, attack the felled timber pine, Pinus sylvestris as a major pest. I. sexdentatus is a European species, introduced into Britain with imported timber and is now commonly found in Southern England attacking the Scots Pine. In bark beetles nematode infection was reported for the first time by Leuckart (1884), and the biology and the life cycle of I. sexdentatus have been studied by Al-Rabiai (1970). Temperature, moisture and continuous air supply were the main factors influencing the subculturing of the beetles (Al-Rabiai, 1970). The nematodes Allantonema mirabile were found to be encapsulated in the haemolymph of Hylobius ebietis. Encapsulated nematodes were also found in Ips typographus (Linstow, 1890). Fuchs (1915) found Tylenchus contortus typographi (Contortylenchus

diplogaster) in I. typographi: they were also encapsulated.

Crofton (1971) has defined the term parasitism in three ways: the state when the parasite injures the host and ultimately causes its death; when the parasite derives benefit from its host and does not kill it; and when there is an equilibrium between the host and the parasite based on evolutionary adaptation, which under suitable conditions ensures continuity of the relationship by the survival of the unharmed host. Salt (1963) stated that parasitism has a considerable effect on host's physiological conditions and development. Brief accounts of the effect of parasitic nematodes on their respective insect hosts have been made by many investigators. Fuchs (1915) considered that Tylenchus contortus (= C. diplogaster) occasionally killed its host I. typographus and in most cases reduced the egg output of the female to about half the normal level. Similar observations were obtained by Massey (1960) in I. confusus infected by C. elongatus. Goodey, (1931) found that there was an effect on the size and fecundity of the parasites in Howardula oscinella, but he was unable to detect any effect on the external characters of the host. Wulker, (1923), never observed any decrease in size in the beetle Hylobius abietis

parasitized by Allantonema mirabile.

Welch (1956) was unable to detect any external effect caused by the parasite, and he found no morphological differences on the head, body or the appendages. Megaselia halterata parasitized by Howardula sp. nematode had swollen abdomens and transparent cuticles; these features have often been noticed (Personal observation, Riding I.L. 1971). The transparency of the cuticle correlated with the diminution of the fat bodies of the host. Diplogasterid nematode infection cause insect mortality as they carry coliform bacteria which caused fatal septicemia in Galleria mellonella (Poinar, 1969).

A significant reduction in the level of glycogen and carbohydrate in the fat body occurred in the adult desert locust Schistocerca gregaria infected with the nematode Mermis nigrescens. Also the carbohydrate in the haemolymph of the males and females was severely depleted during the active growth of the nematode (Gordon et al, 1971; and Gordon and Webster, 1971). Reduction in size, weight and diminution in the fecundity of the host have been reported by Cobb (1921) in cucumber beetles Diabrotica uttata and D. trivittata when infected by Howardula benigna nematode. Massey

(1964, 1965) reported that either no eggs were laid or a drastic reduction in egg production by Scolytus ventralis (Lecout) females and Dendroctonus obesus respectively occurred when parasitized by the nematode Sulphuretylenchus elongatus. Sulphuretylenchus sp. reduced the fertility of I. confusus, (Nickle, 1963). Welch and Bronskill (1962) found that the emergence rate of mosquitoes was retarded by nematode infection. Howardula sp. significantly reduced copulation of M. halterata and multiple parasitism has an adverse effect on the size of both hosts and their parasites (Riding, 1971). Heavily parasitized I. sexdentatus were unable to leave the log, their fat bodies were found to be seriously depleted and they were unable to replace food reserves consumed by large number of parasites in their haemolymph (Al-Rabiai, 1970). Contortylenchus sp. parasitizing I. sexdentatus affected the female's fecundity when the number of eggs laid was reduced by 40%, and the development of infected pupae was also delayed (Al-Rabiai, 1970). The effect of parasitism on the parasites has been recorded by many workers. Goodey (1931) found that there was an effect on the size and fecundity of Howardula oscinellae when they occurred in large numbers. Welch (1956) found the same relation-

ship and "Size - number" relations suggest that there is competition between individuals. Riding (1971) found that as the number of parasites increased, a reduction in length or width of the parasites also increased.

Ruhm (1956) recorded that the rates of parasitism of the nematodes in 12 species of bark beetle sampled, were less than 10%. Massey (1962) gave percentages for different localities with many variations of incidence. Parasitism of fleas with Heterotylenchus pavlovskii increased from a minimum in the spring to the maximum in October (Massey, 1962). Welch (1956) stated that in bark beetles the rates of parasitism of nematodes vary considerably and Al-Rabiai (1970) found no significant differences in the incidence of parasitism between males and females I. sexdentatus infected by Contortylenchus sp.

The life cycle of the bark-boring beetles Ips sexdentatus together with the life cycle of Contortylenchus diplogaster have been established (Al-Rabiai, 1970). This work has been further developed using the various techniques available for the study of the nematode - host relationship.

Host Reaction

Phagocytosis is a phenomenon which is widespread throughout the animal kingdom (Tripp 1963). The defence reactions are usually discussed under the categories of humoral and cellular immunity, especially with regard to insects (Brook, 1969). Humoral factors (antibodies or other factors in the body fluids) and cellular reactions (e.g. phagocytic encapsulation and leucocytosis) form the basis of the host defence. Besides phagocytosis, which is often equated with cellular immunity, encapsulation and leucocytosis are also important cellular defence mechanisms. Most metazoan parasites are too large to be phagocytosed by a single blood cell (Brook 1969).

Encapsulation could be defined as a process of collection and formation of a capsule around a foreign body by cellular elements. Salt (1963) extensively studied encapsulation in insects, and considered the capsules around the parasites to be formed by haemocytes. The resistance of the host to metazoan parasites is attributed to the encapsulating activity of the haemocytes. In his excellent review of an insect's response to a parasite, Salt (1963) reported that defence reactions to metazoan occur in 14 orders of insects, including Lepidoptera, Diptera, Hymenoptera and Coleoptera.

After reviewing the literature, Salt (1963) concluded that the haemocytes of insects were the major defences to the internal metazoan parasite. Shapiro (1969) stated that the host reaction to parasitism depends on the host, the parasite and the environment.

There may be no defence reaction, or the host reaction may retard the growth and development of the parasite, or the reaction may adversely affect a certain percentage of parasites and the reaction may be so effective that the parasite is retarded completely or destroyed (Wittig, 1962);(Doutt, 1963). Host defence reactions can be classified as: reaction of tissues, cells and organelles and as humoral reaction (Salt, 1957).

Several means of defence of insects against the parasite have been listed by Salt (1963), which include that by the virtue of their exoskeleton (actively avoid), passively withstand the attack and then escape infection, destruction by digestive enzymes those parasites that enter through the mouth, and they overcome their internal parasites by various physiological processes. It is chiefly to the latter processes that reactions are made by haemocytes.

The defence reactions to metazoan parasites are generally characterized by the aggregation of the host

haemocytes around the parasite and the deposition of melanin on the parasite surface. Various studies have been carried out on the encapsulation reactions (Salt, 1963, 1970; Poinar, 1969), but how the insect recognizes the foreign body has still not been answered.

Immunity is presented in relation to host escape from (factors which prevent the infection) cellular responses, melanization and humoral properties associated with the arthropod host (Poinar, 1969). The natural immunity of insects to bacteria is mainly phagocytic, but it varies enormously in different insect species with respect to different micro-organisms, (Wigglesworth, 1961). Wigglesworth described the acquired immunity resulting from infection with pathogenic organisms as a general immunity in which the essential response seems to be an increased sensitivity towards the organism on the other part of the body cells, with the nervous system apparently connected in some way with the change. He also believed that phaocytosis, giant cell formation and encapsulation are more motile. Wigglesworth (1961) stated that entomophagous parasites show some specificity to the host in which they will develop, eg. larvae of Loxostege sticticalis are non-susceptible to Eulimnevia sp. (Hymenoptera). This type of specificity may be due to the very active encapsulation by haemocytes. In

invertebrates, Huff (1940) described and divided the mechanisms by which the animals acquire and maintain immunity to infective organisms into two groups dealing separately with humoral and cellular reactions. Cellular immunity or phagocytosis results from the activity of the haemocytes.

Encapsulation of metazoan parasites has been shown to be controlled by haemocytes which play a part in the encystment of the foreign bodies. They form the capsule around the invading body and may cause its death by cutting off supplies of oxygen or nutrients. From past observations which have been made by many investigators, their explanation of the reactions varies considerably according to the cause of immunity of the insects to certain invading parasites. In the last century Metchnikoff, Ceunot, Levaditi and others, noted cellular reactions in insects in response to the invasions of certain parasites.

Marchal (1906) stated that the cyst which surrounded the parasite's eggs was formed by amoebocytes as young conjunctive cells of the host. Pemberton and Willard (1918) found that eggs of hymenopterous parasites deposited within melon fly larvae, become encyted within a mass of transparent cellular material. Holland (1920) discussed the formation of leucocytic agglomeration

around the encysted larvae and that these leucocytes caused the formation of the cyst. The actual defence of the body against infection depends largely on the part played by haemocytes (Wells, 1925). Crickets and grasshoppers have been observed to react to the presence of both nematode and insect parasites. In Acheta (Gryllus) domesticus, the house cricket, Cuenot (1895) found living nematodes, and melanin reactions occurred in an encysted stage, with the cyst surrounded by a covering of haemocytes. Several references to the encapsulation of spiruroid nematodes in the body cavity of grasshoppers were listed by Chabaud (1954). Many authors mentioned the formation of loose capsules around parasites but gave no information regarding its structure. (Fuchs, 1915; Al-Rabiai, 1970). Foster and Johnson (1939) found that in Leucophaea maderae the spiruroid larvae Protospirura muricola was surrounded by a disc-like capsule. The capsules were very thick and a single cockroach often formed several hundreds of them. Salt (1963) mentioned that within 24 hours the parasite was surrounded by a thick capsule with melanin and within two days the parasite was dead. Grimston et al (1967) using the electron microscope studied the cellular capsule formed by the haemocytes of Ephestia kuehniella around foreign objects introduced

into the haemocel; they concluded that foreign bodies implanted in the haemocoel of an insect almost invariably become encapsulated by blood cells. An electronmicroscopical study has been made by Mercer and Nicholas (1966) of the capsule surrounding the acanthocephalan parasite Moniliformis dubius in its intermediate host, the cockroach, Periplaneta americana. They found that the parasites' surface showed features similar to that of the adult host which they interpreted as pinocytes. This capsule does not prevent the growth and development of M. dubius which is still able to take up nutrients through the capsule wall.

It has been suggested by Muldrew (1953), Thorpe (1936) and Wigglesworth (1959) that the capsule inhibits the post-embryonic development of the parasitoid by asphyxiation. Seureau (1973) studied the cellular reactions caused by a subulurid, and spirurid nematodes in Locusta migratoria, its localization and structure. He found that the capsule structure corresponded either to the "granuloma" type haemocytic reaction (subuluridae), to a cellular hypertrophy of the infested tissue with proliferation of fibrillar material (Rictularidae, Physalopteridae, Spiruridae and Dipolotriaenidae), or to a lysis of muscular fibres (Acuariidae).

Wigglesworth (1956) indicated that insect haemocytes contained neutral mucopolysaccharides. The haemocytes secreted neutral mucopolysaccharide during the formation of the capsule of Polymorphus minuta (Crossley, 1964; Crompton, 1964). Bruce (1970) stated that the capsule wall of Trichinella spiralis in host tissue, consisted mainly of collagen and cystine and from an electronmicroscopic study he found that the capsule was packed with mitochondria, rough and smooth endoplasmic reticulum, and golgi complexes. These, together with histochemical reactions, strongly suggested that the capsule's cytoplasm was a region of high metabolic activity. Encapsulation may be quite rapid, occurring in a few hours, or the structure may be changed slowly from one with concentric layers of distinct cells that surround the parasite, to one of a connective-tissue-like syncytium or finally into a thin non-cellular membrane. Many capsules in insects have trachea and tracheoles embedded in their surface tissues - this is probably related to the tendency of these organs to migrate actively towards regions of low oxygen tension (Wigglesworth, 1954).

Fuchs (1915), found that the cellular capsule surrounding C. diplogaster, containing tracheoles and

was covered externally with tracheae.

Encapsulation of parasites has been observed in at least 30 species of coleoptera, chiefly Scarabaeidae and Tenebrionidae. Beetles that have been suggested to encapsulate Spiruroids are listed by Salt (1963). Reports of host reactions in coleoptera were mentioned by Schneider (1871), who observed that Echinorhynchus gigas was enveloped in a fine cellular capsule, composed of connective tissue in the body cavity of the larva of Scarabid Melolontha vulgaris. Kaiser (1893) found that the beetle Cetonia aurata responded to the parasite E. gigas while it was still penetrating through the gut wall.

Some of the earliest and the most thorough work on insect defence reactions made use of coleoptera and was in 1891 when Durham injected indian ink suspended in saline into adult water-beetles Dytiscus marginalis and observed clumps of carbon particles surrounded by haemocytes. Durham mentioned in his paper the essential points of the reaction, the aggregation and flattening of the cells and the formation of a syncytium. An ultrastructural study of the formation of melanotic capsule in the beetle Diabrotica balteata and D. undecimpunctata in response to the mermithid nematode

Filipjevimermis leipsandra has been carried out by Poinar et al (1968). They concluded that their evidence suggests that melanization is an integral and natural consequence of the chance contact of blood cells around the parasite and can be considered as much of a defence reaction as the encapsulation phenomenon. Fuchs (1915) described Tylenchus contortus (Contortylenchus diplogaster) from the body cavity of the adult bark beetles I. typographus and observed that the parasite was often surrounded by a cellular capsule. Ashraf and Berryman (1970) reported that encapsulation of the nematode Sulphuretylenchus elongatus in the body cavity appeared to be surrounded by haemocytes. They observed encapsulated nematodes only in the first to third instars and the reaction occurred only around eggs and first stage S. elongatus larvae. In the present study, an analysis of the cellular reaction of Ips. sexdentatus has been made. Parasitic females of Contortylenchus diplogaster invade the haemolymph of the host, and induce the formation of the cellular capsule, which is derived from host haemocytes. Investigation of the structure of the capsule wall and its content, together with the composition, have been made using the electron and light microscopes and histochemical techniques.

The reaction has also been described in terms of histology and insect physiology.

Contortylenchus (family: Contortylenchidae) was established by Ruhm (1956), as a new genus. He also described many new species and genera including the group of "Contortus" (Fuchs, 1915). Nickle (1967) reviewed the classification of Contortylenchus. Massey (1957) mainly used the variations in morphometry of the adult females of C. brevicomi and C. barberus as a criteria for separating them, while Thong and Webster (1972) redescribed C. brevicomi and C. barberus and synonymized them as C. brevicomi. They believed that the original separation was based on minor morphometric variations, which they considered as intraspecific rather than interspecific.

Nematode parasites from different species of host are not necessarily separate species themselves (Thong and Webster, 1972). Al-Rabiai (1970) recorded Contortylenchus sp. for the first time in I. sexdentatus. She stated that its measurements and characteristics differed from other species, that had been described previously, and suggested that it was a new species without giving sufficient evidence for this suggestion. Fuchs (1930) stated that similar nematodes that occur in

other hosts could belong to the same species, but may have different forms as for example, Parasitaphelenchus sp. in I. sexdentatus, I. acuminatus and Pityogenes bidentatus. Therefore it is possible that the genus of Contortylenchus sp. may have a wide range of related or unrelated host species. Ruhm, (1956), listed species of Contortylenchus spp and their hosts:-

1. Contortylenchus cunicularii (Fuchs, 1929) in Hylastes ater and H. canicularius
2. C. cryphali in Cryphalus piceae
3. C. chalcograph, (Fuchs, 1938) in Pityogenes chalcographus
4. C. tomici in Pityogenes bidentatus and P. quadrideus
5. C. laricis, (Fuchs 1929) in I. laricis and I. suturalis (orthotomicus)
6. C. amitini in I. amitinus
7. C. acuminati in I. acuminatus
8. C. diplogaster, (Fuchs 1915), in I. typographus and I. cembrae

In this study, C. N. sp. Al-Rabiai, was synonymized with C. diplogaster, the reasons for this reclassification are discussed. The variations in the morphometrics of this nematode are related to stresses of the complex host-parasite relationship.

1.2. Physiological aspects of the Host - Parasite Relationship

a) Haemocytes

The haemolymph of insects contains many different types of cellular elements known as haemocytes.

Yeager (1945) divided the blood cells that he found in the caterpillar Prodenia eridonia Cram into ten classes and thirty-two different types. Some of these types had more than two variants. Wigglesworth (1959) and Jones (1962) have provided condensed accounts of more recent work, summarising the various functions of insect blood cell. Jones (1959) restudied the various classes of blood cells in Prodenia using unstained preparations with the phase microscope. This resulted in several changes in Yeager's classification. Jones suggested that this new system would be sufficient for identifying and comparing blood cells in different orders of insects. He found eight classes, and three well defined types are present in the majority of insect species.

In the present study an investigation of the haemocytes of Ips sexdentatus was made. Identification, classification and variations in blood cells pictured at different stages of development of the insects were

determined; following the classification that have been suggested by Yeager (1945), Jones (1959, 1962) and Wigglesworth (1939, 1959). Different types of haemocytes were distinguished by their shapes, type of nucleus, and presence or absence of cytoplasmic inclusions.

Total haemocyte counts (THCs) for different orders of insects have been published by several authors. The THC is a measure of the density of blood cells as a number per cubic millimeter of haemolymph. In some insects, only a few haemocytes may be circulating and others may be attached to the tissue surface as in Rhodnius (Wigglesworth, 1959). Many insects have total haemocyte counts ranging between 20,000 to 100,000 haemocytes per cubic millimeter of blood (Tauber and Yeager 1935, 1936) while others have THCs below 10,000. Numbers of blood cells vary enormously from time to time according to the physiological conditions of the insect and the stage of its life cycle. In the cricket, Gryllus assimilis, the number ranging from 15,000 to 275,000 per cubic millimeter, and average of 70,000 has been obtained, while in Periplaneta americana 15,000 to 60,000 and the highest values were obtained during ecdysis (Wigglesworth 1961).

Shapiro (1967) studied the pathological changes in THCs in Galleria mellonella during the course of Nucleopolyhedrosis and starvation. The THCs decreased in starved and inoculated larvae, and were significantly lower than control larvae. Gilliam and Shimanuti (1967) found that the THCs in honey bees infected with Nosema were fewer than that of healthy bees. In Periplaneta americanus the THCs ranged between 45,000 to 60,000 cells per mm^3 (Wheeler, 1963). Wheeler calculated the number of haemocytes in the entire insect and suggested that this method gave more reliable information concerning changes in the haemocytes number than THCs alone because the latter obviously varied according to the haemolymph volume (Wheeler, 1963).

The increase in number of haemocytes prior to ecdysis is due to a decrease in haemolymph volume. Wittig (1965) studied quantitatively the changes in the blood tissues caused by an insect pathogen in the haemolymph of caterpillars, found that in diseased animals the THCs dropped within a short time to less than half its original value. Nappi and Stoffolano (1972) found that differential haemocytes counts (DHCs) showed a decrease in number of special types of blood cells in Orthellia caesarion larvae infected with the

nematode Heterotylenchus autumnalis. Also in Drosophila melanogaster infected with Pseudeucoila bochei, Nappi (1973) found the DHCs from parasitized larvae showed a slight increase in the percentage of crystal cells and a decrease in the percentage of lamellocytes. He suggested that the parasites had suppressed the immune reactions of the host.

The relationship between different types of haemocytes and the parasite density were determined in this study. Ranges of cells and the nuclear dimensions of all classes of haemocytes are presented. This study attempts to correlate the effect on parasitization of the pupal and adult beetle by Contortylenchus, and the haemocytes involved in the defence reaction.

b) Osmotic regulation

Nematodes which inhabit the alimentary tract of insects are subjected to changes in osmotic pressure, when the host undergoes moulting (Lee 1960) as in Hammerschmidtiella in the intestine of the cockroaches. Like nematodes inhabiting the gut, the parasitic female nematode Contortylenchus diplogaster, inhabiting the haemocoele of Ips sexdentatus is bathed in hypertonic solution.

There is no published data on the osmotic relation-

ship of this parasite to its host, and its isotonicity is unknown. Since nematodes dissected in 0.75% entomological saline burst, this solution was obviously hypotonic. Therefore, experiments were done to determine the effects of several electrolytes and non-electrolytes on Contortylenchus, and to investigate the isotonicity of this worm in which it could survive long exposures. At the same time osmoregulation of the haemocytic capsule was investigated in order to see if the capsule was a living tissue, and to understand the interrelationship between the regulations of the encapsulated nematodes and their capsules.

The osmotic pressure of the haemolymph of insects is generally higher than that of mammalian blood (Florkin and Juniaux, 1964). The osmotic pressure is expressed in terms of a lowered freezing point for insects generally ranging between - 0.5 to 0.9°C. These authors stated that insects are able to regulate the osmotic pressure of their body fluids so that some insects show a considerable increase of the osmotic pressure of the haemolymph during overwintering, owing to the accumulation of glycerol. These changes in osmotic pressure of the haemolymph, will subsequently affect the parasites that live in the blood, thus Contortylenchus diplogaster

in the haemocoel of Ips sexdentatus may need to withstand a fluctuation in the osmotic pressure of the haemolymph.

The Contortylenchus sp. life cycle was described by Al-Rabiai (1970) for the first time in the bark-boring beetle Ips sexdentatus. Only the mature female is parasitic, the free-living infective female penetrates the host pupa (Al-Rabiai, 1970). This female develops and reproduces in the haemocoel of the host, the third stage larvae eventually escaping from the capsule and haemocoel, penetrate the gut and escape from the beetle through the anus as fourth stage larvae, where they start to be free-living.

Contortylenchus lacks a mouth and has no functional gut, so that the body wall and its folds form the surfaces through which nutrients are absorbed. Feeding obviously occurs, since the nematode increases greatly in size after penetration.

2. MATERIALS AND METHODS

2.1. Source of Material

Bark-boring beetles Ips sexdentatus (Boern) were collected from felled logs of the Scots pine Pinus sylvestris from the Forestry Commission station at Yately, Berkshire and Alice Holt Station near Farnham, Surrey, at intervals from April to December throughout the three years of this study.

Several hundred adult beetles, pupae or larvae were obtained from these logs at each time of sampling. Since it was impossible to dissect these insects all at the same time, they were stored alive in plastic containers lined with wet tissue paper with fresh bark at 10°C until required. These beetles were used either for sub-culturing or for further experiments such as incidence of parasitism and encapsulation in wild population at different times of the year from different places. The beetles could be kept for months at this temperature providing a piece of fresh bark was added from time to time.

Hundreds of male and female beetles, from larvae to adults, were dissected from each sample and the data obtained is summarised and illustrated in the results section.

2.2. The Laboratory Maintenance of Ips sexdentatus and the Contortylenchus diplogaster

Al-Rabiai (1970) described rearing techniques for I. sexdentatus but had little apparent success in establishing an optimal supply of insects. The technique used in this study was similar but several slight modifications gave a greater abundance and a more reliable source of material. Freshly cut logs were allowed to dry for a week, then each piece was longitudinally chopped into halves and the two ends and the cut surface were covered with melted wax to retain moisture in the wood. Each piece was inoculated with 4 - 8 male and female beetles, and each log was kept in a polythene bag until the beetles had bored their way into the wood. When frass appeared, the logs were transferred to the 26°C C.T. room, stood in pairs in a tray, covered with a polythene bag, and fixed to the tray to form a seal. Small holes were made in each bag to allow exchange of the gases. Then they were stood on shelves and the whole system was supplied with fresh circulating air at 26°C and relative humidity of 70%. This breeding technique was most efficient because under this condition the beetles emerged within a month or even less regardless of whether they were parasitised or not.

Both infected and uninfected adult beetles were used for sub-culturing in order to maintain the host population and to keep a continuous supply of the parasites. Adult beetles and pupae from each generation were examined to find the incidence of parasitism and for other experimental purposes. Subcultures were made from each generation.

2.3. Haematology

2.3.1. Classification and Identification of host haemocytes

Haemolymph smears were made from insects that had been immersed for 5 - 10 minutes in water at 10°C in order to immobilize the cells. Blood smears were obtained from larvae and pupae by piercing a needle into the side of the insect, leaving it to bleed on a clean slide and then quickly smearing the blood with another slide. Smears were obtained from the adults by severing a proleg and allowing a drop of blood to exude on to a clean slide and smearing as before. Untreated blood smears were also prepared in the same way for all stages in order to see the differences between the two preparations. The smears were allowed to dry in the air fixed in methanol and they were stained in Giemsa stain (Romanowsky modification) for 45-50 minutes. Blood smears for

all stages were examined under a compound microscope and Camera lucida drawings were made for all different classes and types of cells. They were also measured and photographed so that a complete record of haemocytes from the different insect stages was obtained.

2.3.2. Total and Differential Haemocyte Counts (THC and DHC)

THC

Different stages of infected and uninfected male and female Ips sexdentatus were used and the number of parasites in infected hosts varied between 1-10. Blood samples were prepared using a Hamilton 10 µl syringe and 1-2 µl of blood was obtained easily from pupae. To collect the haemolymph the insects were cooled down by keeping them in the refrigerator and then the haemolymph was easily withdrawn, and was quickly diluted 10-20 times using diluting fluid (Feir 1964)(table 1 in the appendix). Samples from adults were obtained from the side of 2nd abdominal segment after the legs were clipped off close to the body. The sample was then mixed well and both chambers of the "Improved Neubauer haemocytometer" were carefully fitted. Blood cells in the ruled area were counted and two counts were made for each sample, the mean number of blood cells was used to calculate the

average number of blood cells per ml. (see appendix for calculation).

DHC

For parasitized and unparasitized insect pupae and imagoes', HDC were made by using well-prepared and stained smears. The strip method of counting was used (Darmady and Davenport, 1968), the cells were counted in strips running from left to right. The first 200 cells were recorded for each count, and two different counts were made from each smear. From those 400 cells, the percentage of each haemocyte type was calculated and the mean percentage of each type from the two counts in every smear was obtained. The data for both parasitized and unparasitized pupae and adult insects was tabulated and illustrated as shown in the results.

2.4. Histochemical Techniques

Infected beetles were dissected, the capsules and the adult parasitic females were collected for histochemical investigation. (Most of the histochemical tests used were as mentioned by Pearse (1968) or otherwise stated). The capsules were immediately fixed in aqueous Bouin, Carnoys, Lillie's buffer, or alcohol. 10% neutral formalin or Baker's formal calcium (Pearse, 1968). Specimens were embedded in 1% plain agar, then dehydrated in graded series of ethanol and graded mixtures of ethanol and celloselve and pure celloselve 2 changes (30 min.) in each solution. The material was then treated with a mixture of celloselve and ester wax and pure ester wax. Sections of 2 mμ thick were cut.

2.4.1. Carbohydrate

Material was fixed in aqueous Bouins, Carnoys and alcohol, and processed as mentioned above, tests used were the Periodic Acid Schiff technique (P.A.S.) to detect glycogen and mucopolysaccharides, Best's Carmine stain for glycogen and toluidine blue for metachromasia. Controls were treated with either diastase to remove glycogen or incubated in filtered salivary amylase for 1 hour at 37°C before staining.

2.4.2. Proteins

The techniques performed on Carnoys, formalin and

alcohol-fixed material were, the mercury bromophenol blue for general proteins, Millon's reaction was used for tyrosine-containing proteins on formalin and alcohol-fixed material.

2.4.3. Lipid

The material was fixed in formalin and the whole nematodes and the capsules were stained in oil red O (Lee 1960a)to demonstrate neutral lipids. Wax sections were stained in Sudan black B to show bound lipid. In control sections lipid, was removed with chloroform:methanol 2:1 (v:v) mixture for 12 hours at 37°C.

2.4.4. Enzymes

Whole nematodes were fixed in cold acetone or cold alcohol for 4 - 6 hours at 4°C or in Baker's formal calcium for 6 hours at 4°C. Specimens were tested for alkaline phosphatase activity using the modified coupling azo dye method (Gomori's technique). Acid phosphatase activity was demonstrated by Gomori's lead nitrate method. Esterase activity was examined using Gomori azo coupling technique. All tests were done with appropriate controls running the same process minus the substrate.

2.4.5. Haemoglobin

Haemoglobin was demonstrated using the Benzidine

method (Cralph's 1941, and Glick 1949). The capsules and the nematodes were fixed in alcohol or formalin for 24 hours. Following this process both the capsule and the nematodes were flooded in benzidine reagent for 15 - 30 minutes, transferred to peroxide reagent for 10 - 15 minutes, washed in distilled water, dehydrated in alcohol then cleared and mounted in Canada balsam.

2.4.6. Amino Acid Analysis

Empty capsules were collected from dissected beetles and kept in diethyl ether in a deep freeze until enough material was available for analysis. The capsules were washed several times in a centrifuge tube with triple distilled water, then the capsules were centrifuged several times with ether, lastly the excess of ether was removed from the final protein fraction, which was hydrolyzed in 4 ml of 6NHCl in a sealed 5 ml ampule. Hydrolysis was in an oven at 110°C for 24 hours. The next morning the acid hydrolysate was filtered and evaporated six times on a watch glass over a steam bath to remove the excess of HCl. Five ml of distilled water was used for each evaporation. The residue containing the amino acids was analyzed in the Biochemistry Department. The amino acids present in the capsule are shown in the results.

2.5. Comparison between the growth of the capsules and the development of the parasite using Feulgen nuclear stain

Encapsulated nematodes were collected from different stages of the host; pupae, young adult males and females and old adult beetles. The capsules were arranged according to the development of the parasites they contained; capsules from pupae contained only immature, developing adult nematodes, capsules from very young adult hosts contain fully mature nematodes and eggs while the third group of capsules contained both nematode eggs and their larvae. In the last group, which were from old adult beetles, the parasite had finished laying eggs, all had hatched and all the larvae had left the capsules. This classification was found to be the most reliable method to follow the development of the capsules in relation to the development of the nematodes they contained.

The staining method of Bird (1972) was used. The capsules were mounted individually on a cavity slide in neutral Canada Balsam under a cover slide. That was the best way to keep the capsules in their shape without any damage, although some distortion was inevitable. Each group of capsules was examined separately with a compound microscope and a Camera lucida drawing was made for

each capsule, for the nematode, and for 10 nuclei chosen randomly from each capsule. The number of nuclei in each capsule was counted and mean number of nuclei was calculated for each group of capsules. Great care was taken to ensure that only single rows of nuclei were counted at a time by avoiding out-of-focus overlapping, the slide was inverted to count the nuclei on the other side of the capsule. The volume of the capsules for each group was calculated and the mean was taken; the same was done for the nuclei. The mean volume of the nuclei was calculated and was multiplied by the mean number of nuclei in each group to give the total volume of nuclei. All volumes were determined using this formula:

$$\text{volume} = \frac{4}{3} \cdot \frac{a^2 b}{8}$$

a = the major axes b = the minor axes

The ratio of the total volume of nuclei to the volume of the capsule for each group was calculated and plotted against the stages of the development of the nematode in each group. The mean number of nuclei in each group of capsules together with the mean volume of the capsules were compared to the developmental stage of the nematode as shown in the results.

2.6. Measurement of Nematodes

During the dissecting of the insects it was noticed that the size of the parasites decreased as the number of parasites present in the haemocoel increased. It was therefore considered important to investigate the effect of parasite density on the parasites. Insects from the same generation and mostly of the same age were collected from the stock culture. In wild beetle populations, normally 3 - 8 adult nematodes were present, and rarely 15 - 18, but as many as 60 females have been found. In the stock culture, higher infections were common and up to 150 females were found in a single insect. The parasites were collected from infected hosts and organized into groups according to the number of parasites present. 1 - 10 nematodes per beetle was most common and 7 - 10 replicated samples in each group were recorded. The measurements of maximum lengths and maximum body widths of parasites were obtained using the ocular micrometer. The volume of individual parasites in each group was calculated according to the method described by Andrassy (1956). The percentage reduction in volume of the parasites was based on the mean volume of a single parasite infection. The percentage reduction in volume from each frequency was plotted against the number of parasites per host.

2.7. Effect of Parasitism on Parasite Fecundity

It was easy to distinguish between eggs and larvae of *Contortylenchus* sp. with a binocular microscope, but was difficult to differentiate between the stages of larvae. It was decided to count the total progeny including eggs for each parasite using a McMaster counting chamber to obtain the total parasite progeny in each beetle rather than differentiate larval stages. The number of adult parasitic female nematodes per host was recorded and the mean number of progeny per parasite was calculated. From this data the percentage reduction in number of progeny per parasite in relation to the number of adult female parasites per host was obtained using single infections as controls. The data was summarised and the percentage reduction in the mean progeny was plotted against the number of adult female parasites per host.

2.8. Effect of Starvation on Heavily Infected Host

One hundred adult male and female *I. sexdentatus* from the same generation were collected from a laboratory stock culture and were used in starvation experiments. The beetles were kept in plastic containers lined with wet tissues covered with black filter paper and kept in black polythene bags without food at room temperature.

The beetles were left for 4 weeks and checked every day for mortality. Dead beetles were dissected to determine the infection rate and arranged in groups according to the number of parasites per host. The data was summarised then expressed against the mortality time as shown in the results.

2.9. Osmoregulation Experiments

2.9.1. For adult parasitic nematodes free in the haemolymph

Adults of I. sexdentatus were dissected in 0.85 per cent entomological saline. Adult parasitic females of Contortylenchus were collected from the haemolymph of the host and used in the experiments because they were not so active as the larvae and were therefore easier to measure.

The nematodes were individually placed in various experimental solutions in solid watch glasses filled with the test solutions. The watch glasses were covered with glass and sealed, the length of each nematode was measured in 0.85 per cent entomological saline and then placed in test solution. The watch glasses were kept covered in perspex dishes with tap water to keep the humidity constant. The nematodes were observed and measured under a microscope using a micrometer eyepiece

at 30 min. intervals (Croll & Viglierchio 1969). This measured the increase or decrease in length in various solutions. Five individuals were used and the changes were expressed as the mean percentage change.

The solutions used were various dilutions of entomological saline, different molarities of sodium chloride and sucrose and distilled water.

2.9.2. Effect of changes in the osmotic pressure upon the capsules of Contortylenchus

Infected pupae were dissected in 0.85 per cent entomological saline and the capsules were placed in 0.85 per cent in cavity slides. Initially, drawings were made using Camera lucida for each capsule, and the capsules were transferred to another cavity slide with experimental solutions, Camera lucida drawings were made for each capsule at 30 min. intervals. The drawings were made on the same kind of paper, then these drawings were cut and weighed, and the differences in the weight of the paper were calculated. Capsules in a cavity slide were covered with a coverslip, sealed, and placed on a bridge in a covered perspex box containing a little water at the bottom to prevent evaporation. Four capsules were used for each experiment and all the capsules were collected from pupae because they were easier to see. The solutions

used were various molarities of sodium chloride and different dilutions of artificial sea water.

2.10. Oxygen consumption rate

1. Adult beetles

I. sexdentatus of the same age and the same generation from a lab. culture were used. There were equal numbers of infected and uninfected male and females, but the infected ones had different numbers of parasites. One insect was used for each examination in a Spenser Davis (1966) constant pressure respirometer. (see appendix). Each individual insect was allowed to respire for between one to two hours until a rate was obtained. The respirometer was then removed from the water bath, and the fluid displaced in the manometer was measured, using a micrometer. The oxygen volume used during that period was then calculated (see appendix). At the end of each experiment the insect was dissected, and the number of parasites recorded. The tissue of the host and the parasites were bulked dried then freeze dried for 24 hrs. (using Edward's freeze dryer) and weighed using a micro-electron pan balance. The amount of the oxygen consumed was calculated in $\mu l O_2/\mu g$ d. wt./hour, for each parasite frequency group of males and female beetles (appendix 4) The data was summarized and illustrated as shown in the results.

2. Adult Parasitic female nematodes

The respiratory rate of the parasitic females Contortylenchus sp. was determined with an oxygen electrode. (Yellow Springs Instrument Co. ICN models 53), biological oxygen monitor. Beetles from the same generation were dissected and nematodes found free in the haemolymph were collected. Forty to eighty individuals were used at a time, each group of nematodes were from a single host. More than one experiment was done using the nematodes which were collected from a single host when it was heavily infested by more than 100 adult parasitic female nematodes. Active, undamaged adult nematodes were counted and transferred by a fine hair to the cylindrical respiration vessel which contained a known volume, of 0.85% entomological saline and a magnetic stirrer. To avoid damage to the nematode the magnetic was extremely small. The electrode was standardised with distilled water passed through a rubber plunger which fitted tightly into the neck of the respiration vessel. A slot in the side of the plunger allowed the air to escape when the electrode was pushed into the vessel. The electrode was standardised with distilled water saturated with air, a probe test was performed at the beginning of the experiments daily. If the drift was more than 0.5 per cent in

15 minutes the membrane was replaced. The experiments were carried out at 30°C. The nematodes were left to respire for 10 - 20 minutes, then removed and washed with distilled water and centrifuged several times, before freezing and drying. The dry weight of each group of nematodes was measured using a microelectron balance as previously described. The amount of dissolved oxygen per ml of entomological saline under one atmospheric pressure at 30°C was obtained from Manometric techniques of Umbreit et al (1957) for 0.0260 ml of O_2 is present in 1 ml entomological saline. From that, the amount of oxygen consumed by nematodes and in the experimental solution used was calculated. By recording the percentage saturation of oxygen at the beginning and at the end of the experiments in a known period of time the difference between the two gave the percentage of oxygen consumed by the nematodes in that time. After obtaining the dry weight of the nematodes the amount of oxygen in µl per 1 µg dry weight in 1 hour was calculated as shown in the results. After running the experiment for 15 minutes 10 µl of 0.1 M potassium cyanide was added as an inhibitor and the experiment rerun for 15 minutes then the nematodes were treated as previously described, in 10 µl of 0.1 M KCN there was 6×10^{-4} mg KCN.

2.11. Electron Microscopy

Female Contortylenchus and capsules were collected from different stages of Ips sexdentatus from freshly dissected insects with a fine pipette. They were immediately immersed in 2.5% cold gluteraldehyde in 0.05 M cacodylate buffer at pH 7.2 with added 2% calcium chloride for 4 hours at 4°C. Fixation was followed by several washes with cacodylate buffer containing 7.5% sucrose (Gordon et al 1963).

Post fixation in Millonig's (1961) was used with phosphate buffered Osmium tetroxide for 3 - 4 hours at 4°C. After 2 hours in gluteraldehyde the capsules were pierced to facilitate the penetration of the fixative. The nematodes within the capsules were well fixed even when the capsules were not pierced. Dehydration was carried out through graded alcohols; the absolute ethanol used was water-free by using anhydrous copper sulphate and it was filtered before use, the specimens were left 1 hour in each change up to absolute alcohol at 4°C, then the process was carried out at room temperature with two changes of absolute alcohol.

The TAAB resin was freshly prepared prior to use. Portions were vigorously shaken with absolute ethanol to provide a series of increasing concentration from

alcohol to pure resin. The capsules and the nematodes were passed up this series for about 30 minutes in each mixture, until they were transferred to pure resin and left overnight exposed to continuous agitation. They were transferred to fresh resin in a TAAB capsule the next morning and polymerization took place with 2 - 3 days at 45°C. The resin mixture which gave the most satisfactory results was the combination of 10 parts resin : 7 parts DDSA (Dodecenylsuccinic anhydride hardener) : 3 parts MNA (Methyl nadic anhydride).

Sections were cut with glass knives made on an LKB ultra electron-microtome. Pale golden or silver grey interference coloured sections were usually collected on uncoated 100 micron copper grids. Grids had been previously dipped into a cellotape-chloroform mixture to ensure adherance of sections. Staining was done with uranyl acetate (Watson 1958) followed by lead citrate, then rinsed with 0.02 N sodium hydroxide. Thick sections were also collected on thin glass coverslips and stained with 1% toluidine blue in 1% borax sclution for examination under a light microscope. Electron microscope observations were made with Philips Em300 electron microscope at 60 and 80Kv.

3. RESULTS

3.1. Ips sexdentatus Life Cycle

Adult Ips sexdentatus is the largest species of its genus (Plate 1) varying in size between 4.5 - 6.0 mm, it is dark brown or black in colour. The beetle takes about a month or less in the laboratory from adult to adult at 26°C. The eggs hatch in about 2 - 3 days, and larvae pass through several instars in 10 - 15 days. The last instar to a pupa from which adult beetles emerge occurs after 2 - 3 days in healthy animals, but infected beetles may take about 5 - 7 days to develop into adults.

PLATE 1

LIFE CYCLE OF IPS SEXDENTATUS, PINE BARK-BORING BEETLE.

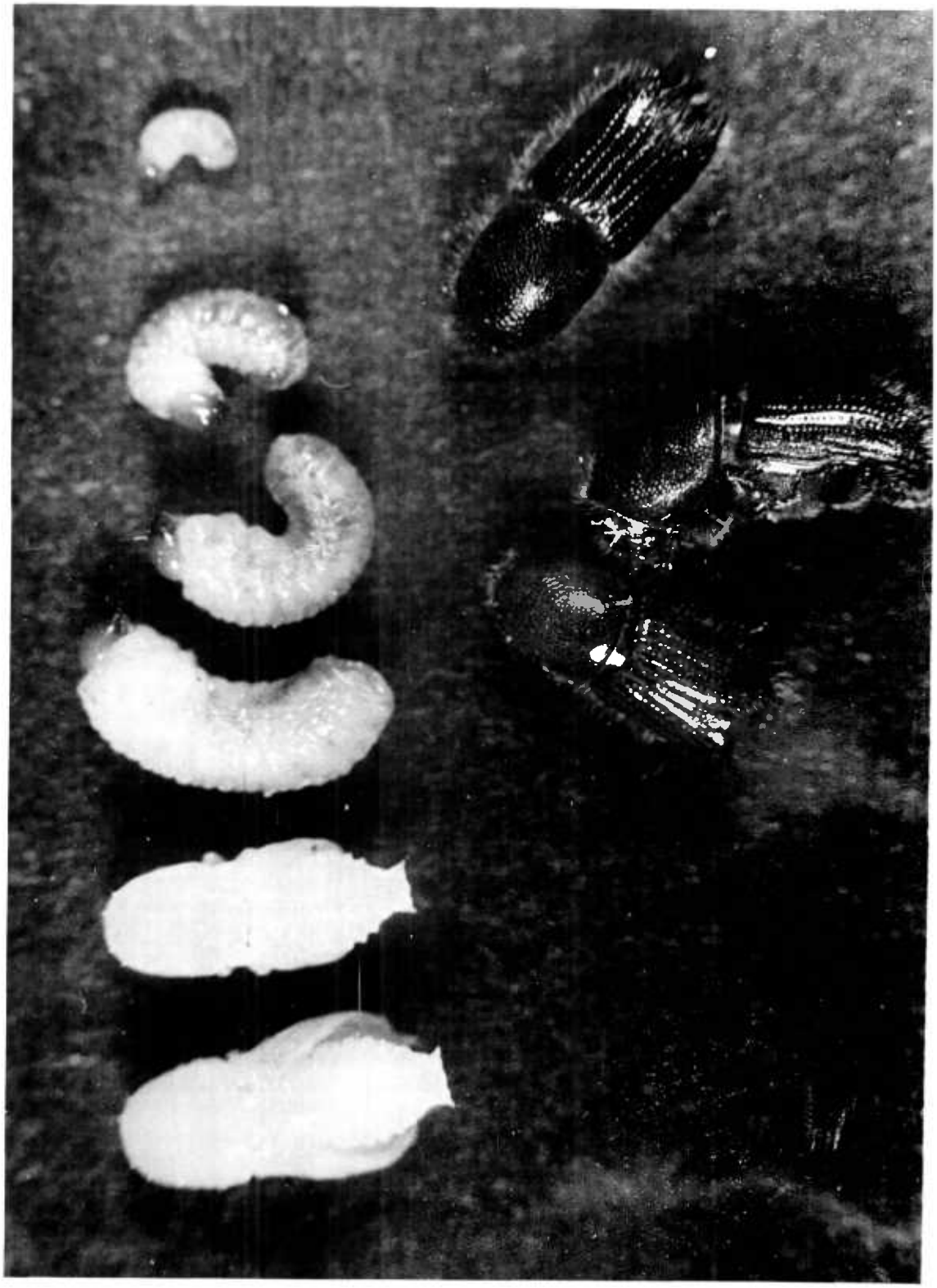
First row from top to bottom

Four instars larvae, Pupa and pre-adult stage.

Second row from top to bottom

Newly emerged adult, female adult and male adult beetles.

(X 10)



3.2. Classification of the haemocytes in different developmental stages of the beetles, I. sexdentatus

Different types of haemocytes were distinguished, firstly on the basis of their shape, type of nucleus and presence or absence of cytoplasmic inclusions. Secondly, following the classification which has been suggested by Jones (1962) which is very similar to Wigglesworth's (1939), the following types of haemocytes were found:

1. Prohaemocytes

Small spherical cells with relatively little pale or dark grey basophilic cytoplasm, they were the smaller cells in the haemolymph with a large rounded nucleus and were often seen in mitosis (Fig. 1, A). They measured between 7.2 µm to 24 µm in diameter. The nucleus was surrounded by a clear zone, probably because the nucleus had shrunk in relation to the cytoplasm. They were found in large aggregates and were numerous in the larval stages. It was thought that they were to be young, developing cells and considered by several workers to be the stem cells from which some, or all, other types derive, such as plasmatocytes, granular haemocytes, spherule cells, and others. They are less abundant in pupae and there are very few in adults. These cells were similar to those

described by Yeager (1945) and Wigglesworth (1939) where they called them preleucocytes (Rooseboom 1937) (Fig. 1A)

2. Plasmatocytes

These were large, highly pleomorphic cells usually with a single large centrally located nucleus in an abundant, mostly basophilic and finely granulated cytoplasm. They were the dominant type in the haemolymph of the insects, and may be the principle encapsulating cells. They were able to send out cytoplasmic extensions, and were polymorphic, having various irregular shapes; fusiform, spindle, pointed ends, or one end true spindle and the other rounded or ovoid. Some of them possessed cytoplasm differentiated into ectoplasm and endoplasm. The ectoplasm appeared thinner and was less intensely stained than endoplasm, some of these cells were multiramous but they are very rare and were found only in adult and pupae in few numbers. These cells were found in all stages but they differed in the degree of vacuolization of the cytoplasm. In pupal hosts they were highly vacuolated, and each individual vacuole appeared circular (Fig. 2B). In the adults these plasmotocytes are the largest cells and are more irregular (Fig. 2B). They have usually rounded or ovoid punctated nuclei. The nuclei were different in different cells or sometimes in the

FIG. 1

TYPES OF HAEMOCYTES IN IPS SEXDENTATUS, PUPAE AND ADULTS.

Key

A. Prohaemocytes.

G. Granular haemocytes.

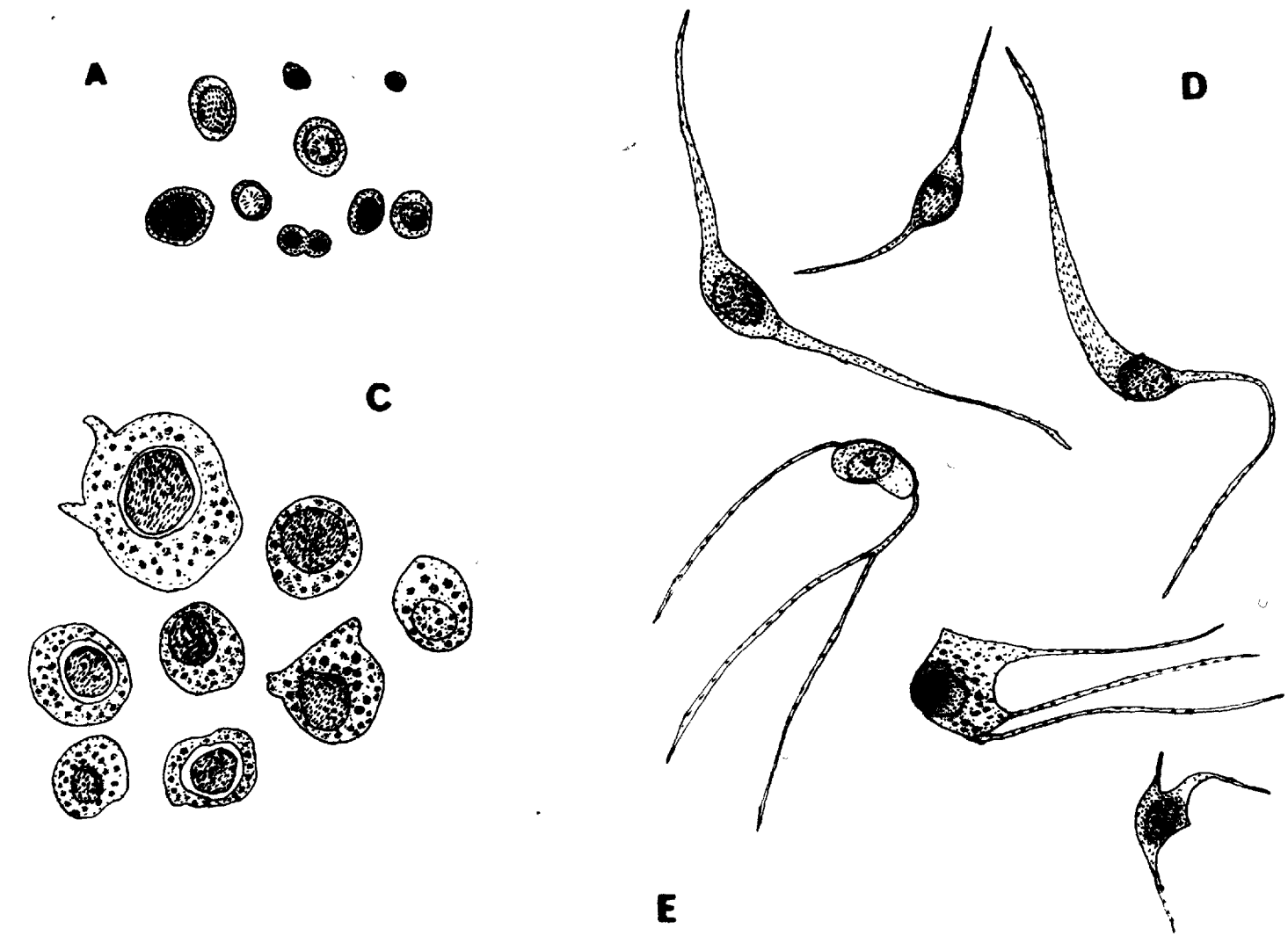
D. Vermiform cells.

E. Multiramous vermiform cell.

The scale for all the Haemocytes is

48 µm

FIG.



same cell. According to Yeager (1945) the vacuoles correspond to the glycogen inclusions. He also divided the plasmatocytes, according to their size, into micro, meso and macro plasmatocytes, and according to their shape into elongated multiramous forms which had more than two spindle ends (Fig. 2B). The diameter of these cells varied between 12 µm x 33.6 µm to 103 µm x 72 µm.

3. Granular haemocytes

These were big cells containing uniform sized, intensely acidorhilic inclusions, with a centrally located nucleus surrounded by a clear zone. These granules were more abundant than in plasmatocytes. They were derived from prohaemocytes or possibly from plasmatocytes. They were able to send out pseudopodia. Jones (1956) suggested that these cells which send out pseudopodia are phagocytic as in Sarcophaga and Prodenia Yeager (1945). They measured between 26.4 µm to 55.2 x 60 µm and were found in larvae, pupae and adult (Fig. 1C).

4. Vermiform cells (worm like, Yeager 1945)

These were elongated, fusiform, thin and thread like cells measuring between 62 x 33.7 µm to 108 x 15 µm (Fig. 1 D). They were derived from plasmatocytes. Yeager (1945) suggested that they might be derived from elongated plasmatocytes, they had centrally located, ovoid

or punctate nuclei. They had light basophilic cytoplasm, their spindle ends become very fine and indistinct at the ends. These cells were rarely seen in insects, (Jones, 1962) but they were found in all stages of this insect.

5. Multiramous vermiform cells

These cells were similar to vermiform cells but they were found with three instead of two spindle ends. They were found in adults and pupae and had a light basophilic cytoplasm and relatively big centrally located nucleus (Fig. 1 E).

6. Podocytes

These were large, thin and flat cells, with three to eight long pointed cytoplasmic extensions and a few colourless vacuoles. Some of these were fusiform with long spindle ends, but in addition they had one or more shorter cytoplasmic extensions, they measured between 48 x 12 µm to 156 x 24 µm. They were found in larvae, pupae but were more common in adults (Fig. 3 F).

7. Spheriodocytes

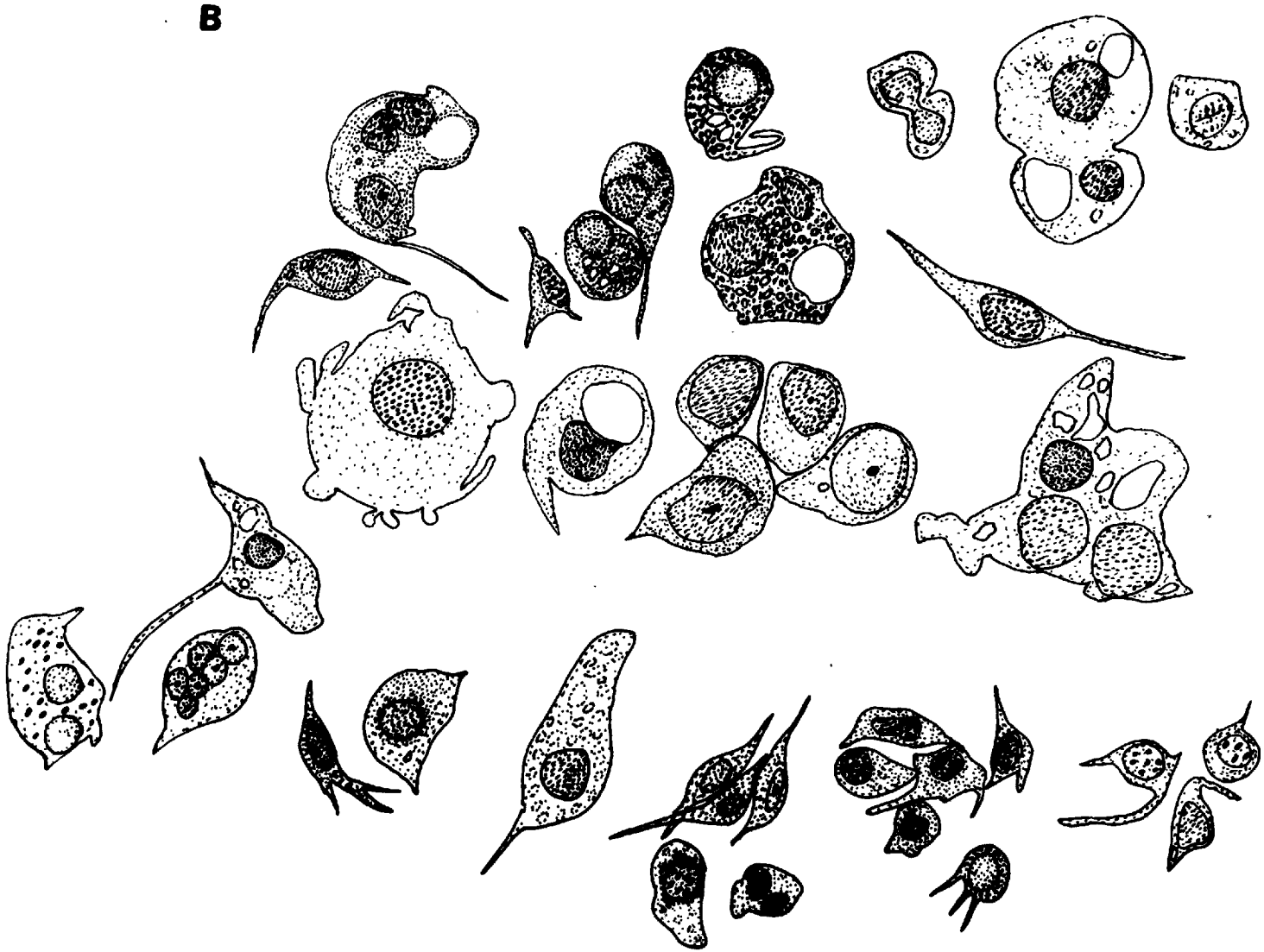
These haemocytes were rounded, ovoid or irregular in shape with large distinct acidophilic spherular, and coarsly granulated basophilic cytoplasm, and some times were highly vacuolated. These cells have centrally or

FIG. 2

B. PLASMATOCYTE OF IPS SEXDENTATUS, PUPAE AND ADULT.

Some of the cells appeared in process of division

(Scale 48 µm)


B

eccentrically located punctate nuclei, the nuclei were sometimes covered with the acidophilic spherular so it becomes indistinct; when the spherular breaks down, the nucleus becomes visible and the cytoplasm becomes vacuolated and granulated (Jones 1962). Dennell (1947) stated that these spherular contain tyrosinase in crystals, the cells vary in size between 19.2 x 26.4 µm to 64.8 x 55.2 µm. They were found in all stages (Fig. 3 G) of the beetles.

8. Oenocytoids

These were large, thick cells, with different shapes. They were relatively small, rounded, grossly punctated with one or two eccentric nuclei, the cytoplasm was great in amount and highly basophilic, with fine granules or rod-like crystals. Decleir et al (1960) and Riziki (1959) suggested that these crystals contain tyrosine, these cells break down very easily and release their material, they were found in pupae and in the adult (Fig. 3 H) and they measured between 36 µm x 12 µm to 60 µm x 36 µm.

9. Amoebocytes

These cells are found to be the most abundant type of cells in the haemolymph of adults and pupae. They were pleomorphic, rounded, spindle shaped or irregular, they had pseudopodia. They were probably derived from plasma-

FIG. 3

TYPES OF HAEMOCYTES IN IPS SEXDENTATUS PUPAE AND ADULTS.

Key

F. Podocytes

G. Spherule cells

H. Oenocytoid

I. Different shapes of Amoebocytes
Some appeared multinucleate cell.

J. Hyaline cells (eruptive)

(scale 48 µm)

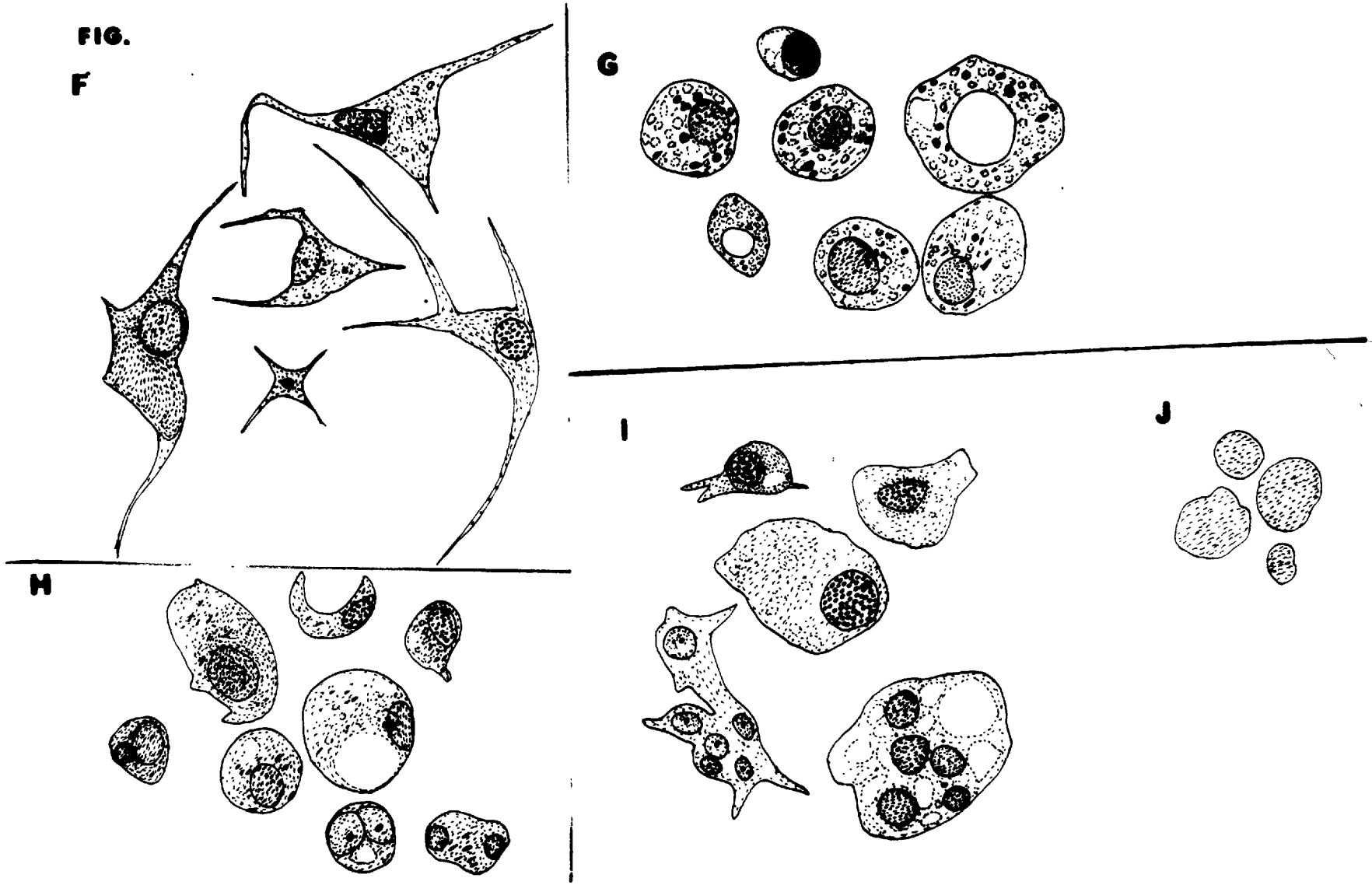


FIG.
F
G
H
I
J

tocytes, they were the most active phagocytic cells because of thin filamentous pseudopodia, they were multinucleate with basophilic cytoplasm. They were very big and highly vacuolated in the adult stages. Amoebocytes were studied thoroughly by Wigglesworth (1956); he suggested that they were composed of mucopolysaccharide material, they measured between 21.6 µm x 36 µm to 60 µm x 76.8 µm and were found in pupae and adults (Fig. 3 I).

10. Hyaline cells

These were cells with acidophilic cytoplasm, finally granulated and with indistinct nuclei. They usually measured between 9.6 µm and 28.8 µm in diameter. They resembled the eruptive or degenerative cells of Yeager (1945) (Fig. 3 J).

3.3.1. Total haemocytes counts (THCs) of unparasitized pupae and adults

Values of THCs of uninfected pupae and adult bark beetles are shown in Table 1. The total counts were much higher in the pupae than in the adults and they were also higher in male pupae and adults than in female pupae and adults respectively, although the difference was only significant in the adult males.

Table 1 THCs of unparasitized male and female pupae and adult beetles

Stages and sex	No. of insects examined	No. of blood cells/cubic mm of blood		
		Range 10^3	Mean	± S.E.
Pupa				
Male	10	141.6-190.4	170,583	4.28
Female	10	149.5-195.8	166,732	4.09
Adult				
Male	16	76.2-203.2	125,726	16.46
Female	15	67.0-165.6	96,856	7.22

3.3.2. DHCs for uninfected and infected pupae and adult host

The terminology suggested by Jones (1962) has been

adapted in the present study for blood cell and basing originally on Yeager's classification (1945) with slight modification according to the findings. So the following haemocytes types were distinguished in this insect: Prohaemocyte, Plasmatocyte, Podocyte, Spheriodocytes, Vermiform cells, Oenocytoid, Ameobocytes, Granular haemocytes and degenerated cells.

The Plasmatocytes constituted the largest proportion of the total haemocytes in beetles, then followed by spheriodocytes, but the prohaemocytes were numerous only in developing instar stages. In DHCs most of the cells were believed to be derived from plasmatocytes and were included with plasmatocytes, although technically this was incorrect. The DHCs of infected host were completely different from those of uninfected beetles (table 2, 3). These differences occurred as a result of pathological changes to insect haemocytes resulting from their immune defence reaction against the invading parasites, then histological changes occurred (DHCs).

Table 2 DHCs of unparasitized larvae, pupae and adults

Stage of the host	No. of cells counted	Percentage of different kinds of cells counted			
		Prohaem-ocytes	Plasmat-ocytes	Spher-ules	Vaculo-cytes
4th. instar larvae	400	50.5	17.75	25.75	6
Pupae	400	49.25	18.5	13.7	18.5
Adults	400	11	63.5	18.75	8.25

Table 3 DHCs of parasitized Ips sexdentatus pupae and adult with Contortylenchus nematode

Stage of the host	Total no. hae-mocytes counted	Percentage of different kinds of cells counted			
		Prohaem-ocytes	Plasmat-ocytes	Spher-ules	Vaculo-cytes
Pupae	400	57.00	15.75	16.25	11.00
Adults	400	34.75	33.00	13.5	18.75

THCs of infected hosts (pupae and adult beetles)

The THCs of male and female pupae infected with Contortylenchus were far less than the unparasitized ones

and the THCs decreased according to the parasite density. Eighty percent reduction was obtained in total haemocytes from adults and up to 40 percent reduction form pupae, although the THCs for unparasitized pupae were much higher than unparasitized adults. There was no significant difference between the THCs of infected male and female adults so they were combined in one line.

There was a linear relationship between the percentage reduction of total haemocytes in beetles as the parasite burden in the haemocoel increased (Fig. 4). The value of F for (1,12) degrees of freedom was significant P = 0.05.

THCs for infected male and female pupae

There were significant differences between male and female pupae by a Student's "t" test, giving a significant "t" value for 10 degrees of freedom P = 2% + 1% (see appendix for calculation) so they were separated on different lines.

There was again a linear relationship between the percentage reduction in the total number of haemocytes in male and female pupae as the parasite burden increased (Fig. 5). The F value for (1,5) degrees of freedom was significant, P = 0.05, since the 5% critical value of F for (1,5) is 6.61 (Appendix 5).

A linear relationship between parasite density and

FIG. 4

EFFECT OF PARASITE DENSITY ON THE TOTAL HAEMOCYTE COUNTS OF ADULT MALES AND FEMALES BEETLES.

Key

◆-◆ . . Adult females

◇-◇ . . Adult males

○ -○- .... equation points

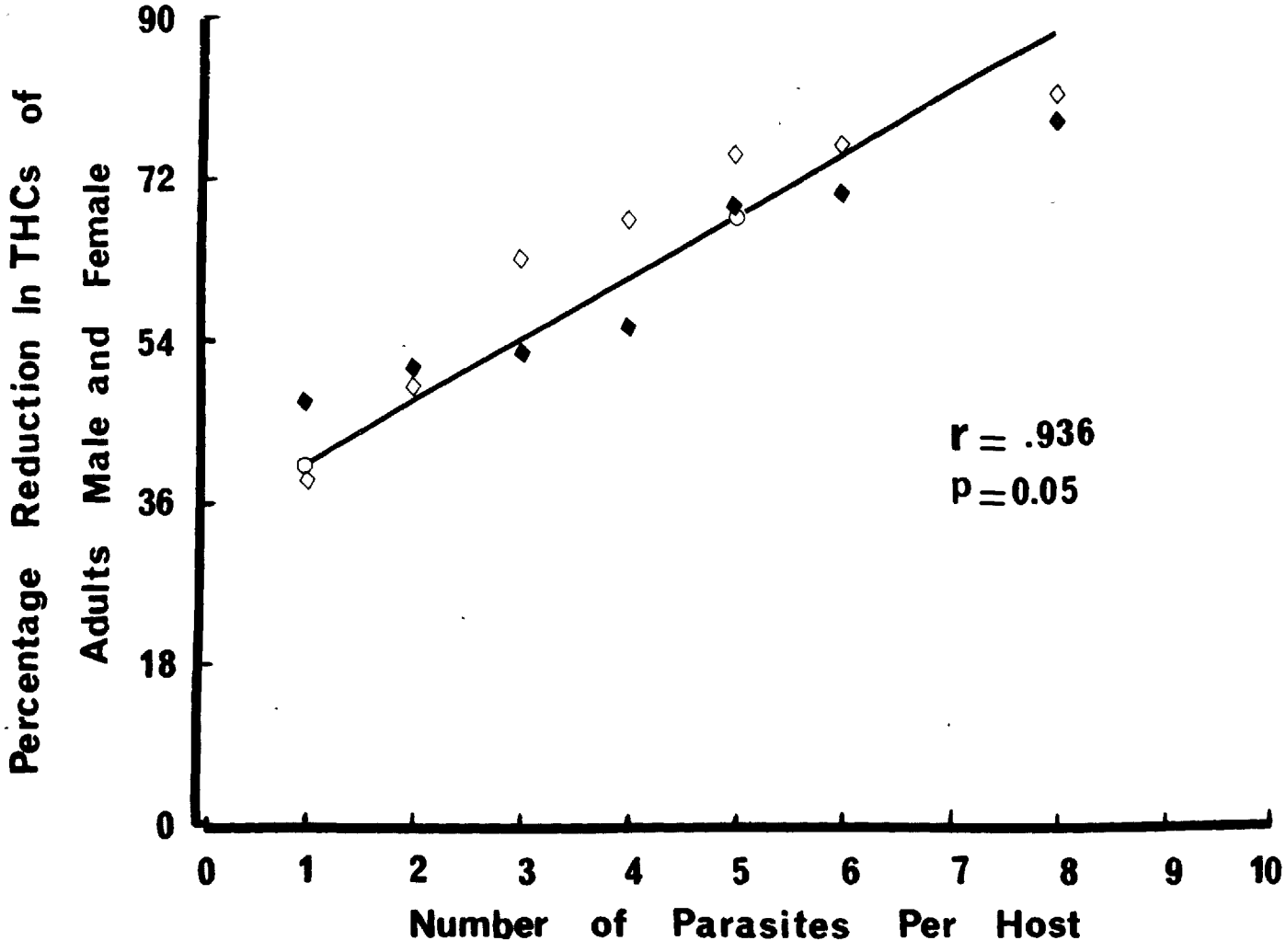

Percentage Reduction In THCs of Adults Male and Female
90
72
54
36
18
0
0
1
2
3
4
5
6
7
8
9
10
Number of Parasites Per Host
r = .936
p = 0.05

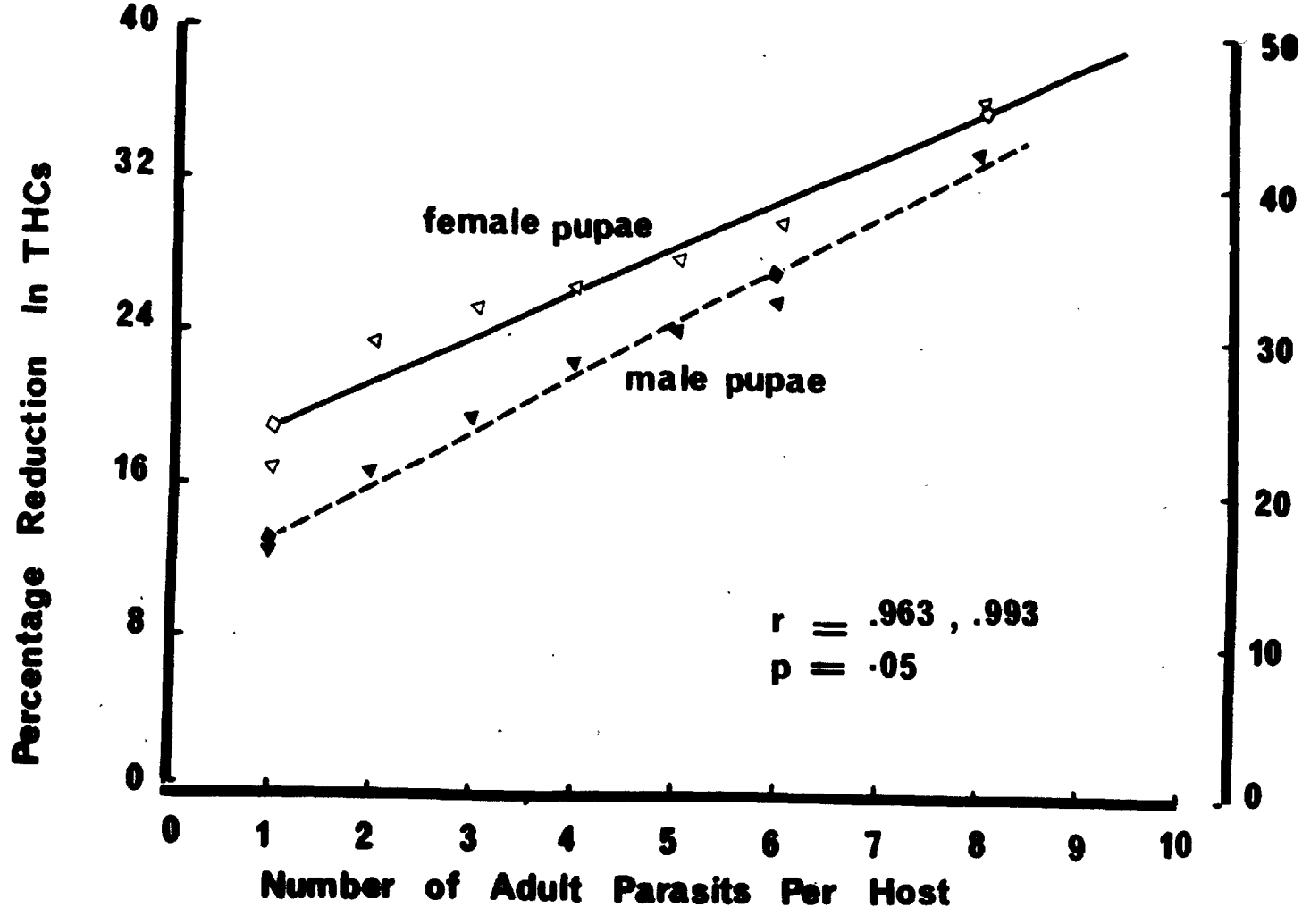
FIG. 5

EFFECT OF PARASITE DENSITY ON THE TOTAL HAEMOCYTE COUNTS OF MALE AND FEMALE PUPAE

Key

◇·◇··· ····· equation points for female pupae

◆·◆··· ····· equation points for male pupae


Percentage Reduction In THCs
40
32
24
16
8
0
female pupae
male pupae
r = .963 , .993
p = ·05
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Number of Adult Parasits Per Host

percentage reduction in THCs of infected male pupae (Fig. 5). The F value (1,5) was also significant P = 0.05, since the 5% critical value of F for (1,5) is 230.2 (Appendix 5).

3.4. The initial role of haemocytes in capsule formation and its subsequent development

Invasion of bark-boring beetles Ips sexdentatus by infective female Contortylenchus commenced in the pupal stage. A physiological defence reaction by the pupal host took place soon after invasion. This reaction commenced with an aggregation of the host's haemocytes around the invading parasite forming a capsule. The capsules are found in one day old pupae but neither nematode or capsule were present in earlier instars. Hundreds of larvae were examined during this study but no infection was recorded in any instars. All these observations were done under light microscope after dissection.

In the pupal host, unencapsulated nematodes were never found regardless of the parasite's density in the host. The parasite enters the host's haemolymph and since it was composed foreign proteins, the haemocytes were stimulated in some way to move in large numbers and surround the parasite, flattening themselves against it to form the cellular capsule. After formation of this envelope, the reaction continued as the parasites developed, more haemocytes attaching themselves to the surface of the envelope.

3.4.1. - 3.4.4. The ultrastructure of the capsule from pupal and adult host

Initial observations on the fine structure of the capsule, showed that its structure could be related to the blood cells from which it was derived. In this study an attempt to investigate in more detail the different types of blood cells chiefly concerned with capsule formation and development was undertaken using the electron microscope.

The haemocytes involved in the encapsulation reaction were mainly polymorphic plasmatocytes, amoebocytes and granular haemocytes. These cells have the ability of phagocytosis and produced cytoplasmic extensions. Plate 2 typifies the kind of haemocytes which were more common in the haemolymph and associated with the capsule. Fine pseudopodia can be seen extended from their surfaces. (see Plate 8) The cytoplasm of the cells filled with different kinds of organelles and many numerous vacuoles about 0.15 - 0.25 µm in diameter, some of these vacuoles can be seen in the process of formation on the cell surface, they are possibly formed by pinocytosis. In addition there are small tubules and vesicles lying between other big organelles. Cytoplasmic membranes were found with Golgi bodies in the form of vesicles. Free ribosomes were found in abundance.

PLATE 2

HAEMOCYTES INVOLVED IN CAPSULE FORMATION

Key

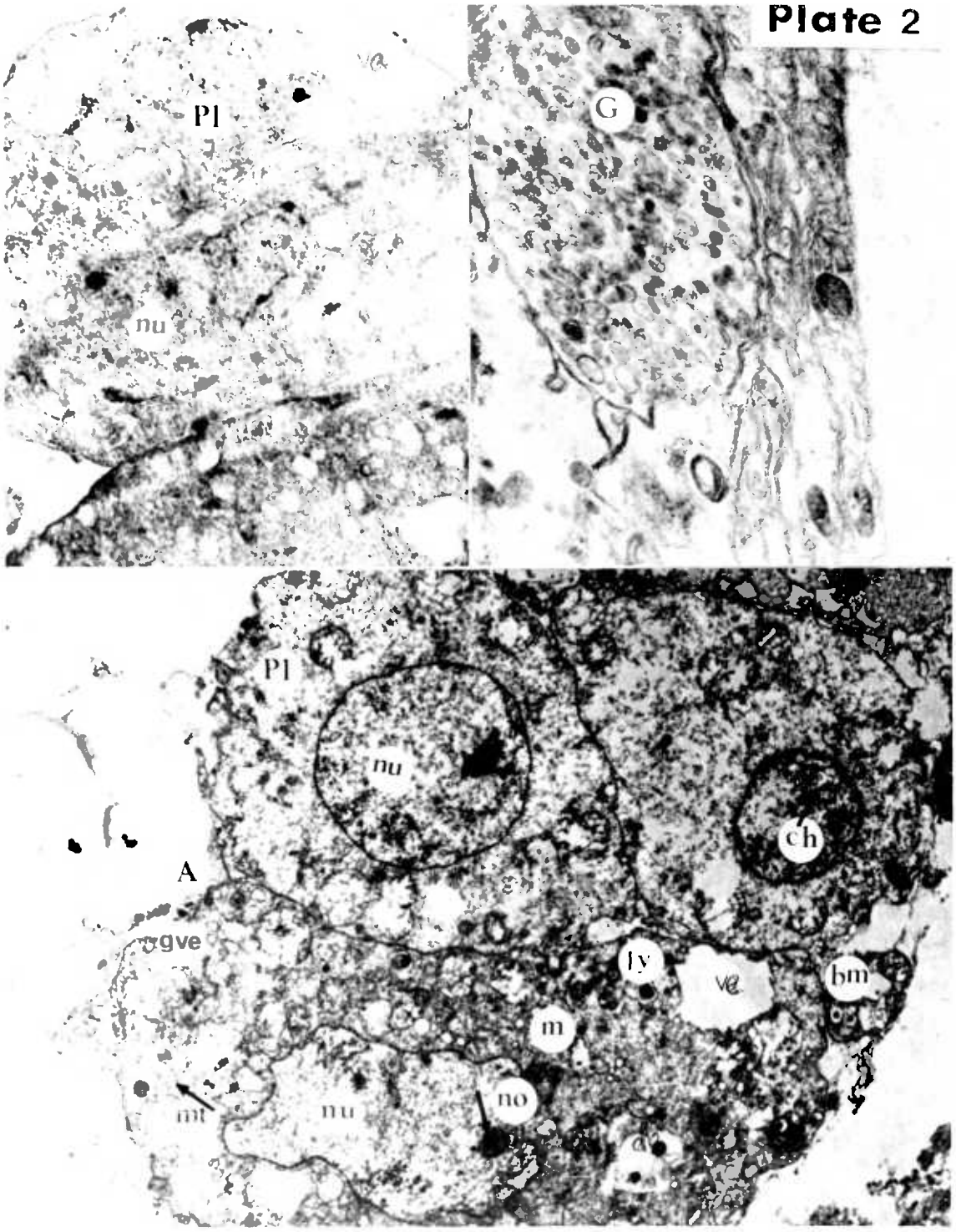
nu	nucleus
no	nucleolus (arrow)
ch	chromatin (arrow)
m	mitochondria
bm	branched mitochondria
va	vacuoles
ve	vesicles
db	small dense bodies (lysosomes)
mt	microtubules (arrow)
gve	golg. vesicles
A.	Amoebocytes
PL.	Plasmatocytes
G.	Granular haemocytes

(X 27360) for Granular haemocytes top right

(X 12148.5) for Plasmatocytes top left

(X 5803.6) for Amoebocytes bottom

Plate 2



Microtubules were found prominent in all cells. The cytoplasm of these haemocytes contains a typical mitochondria, sometimes being branched and containing dense inclusions (Plate 2). Small spherical dense bodies ranging between 0.05 - 0.1 μm in diameter were found in almost all blood cells (Plate 2) which were identified as lysosomes.

The haemocytes congregate (Plates 2, 3) about the nematodes, become flattened and the cell membranes break down to form the capsule. Contact between cells outside the capsule was seen (Plate 5). These cells were joining the capsules externally, the cell membranes break down at the point of contact, are lysed and then merged their contents with the capsule's cytoplasm (Plate 5). The capsules appeared multinucleate through the fusion of the cell contents, nuclei fusion was observed (Plate 29B). The haemocytes congregate and attach themselves to the capsule externally, while the reaction still continued and the parasite within the capsule develops and reproduces. These cells have normal nuclei and still retain their original shape (Plate 2). During this phase, the capsule increases in thickness. It acquiries spherical shape and smooth surface when no more blood cells were being added, at that time the nematodes progeny start to leave the

PLATE 3

DIFFERENT TYPES OF HAEMOCYTES INVOLVED IN CAPSULE FORMATION

Key

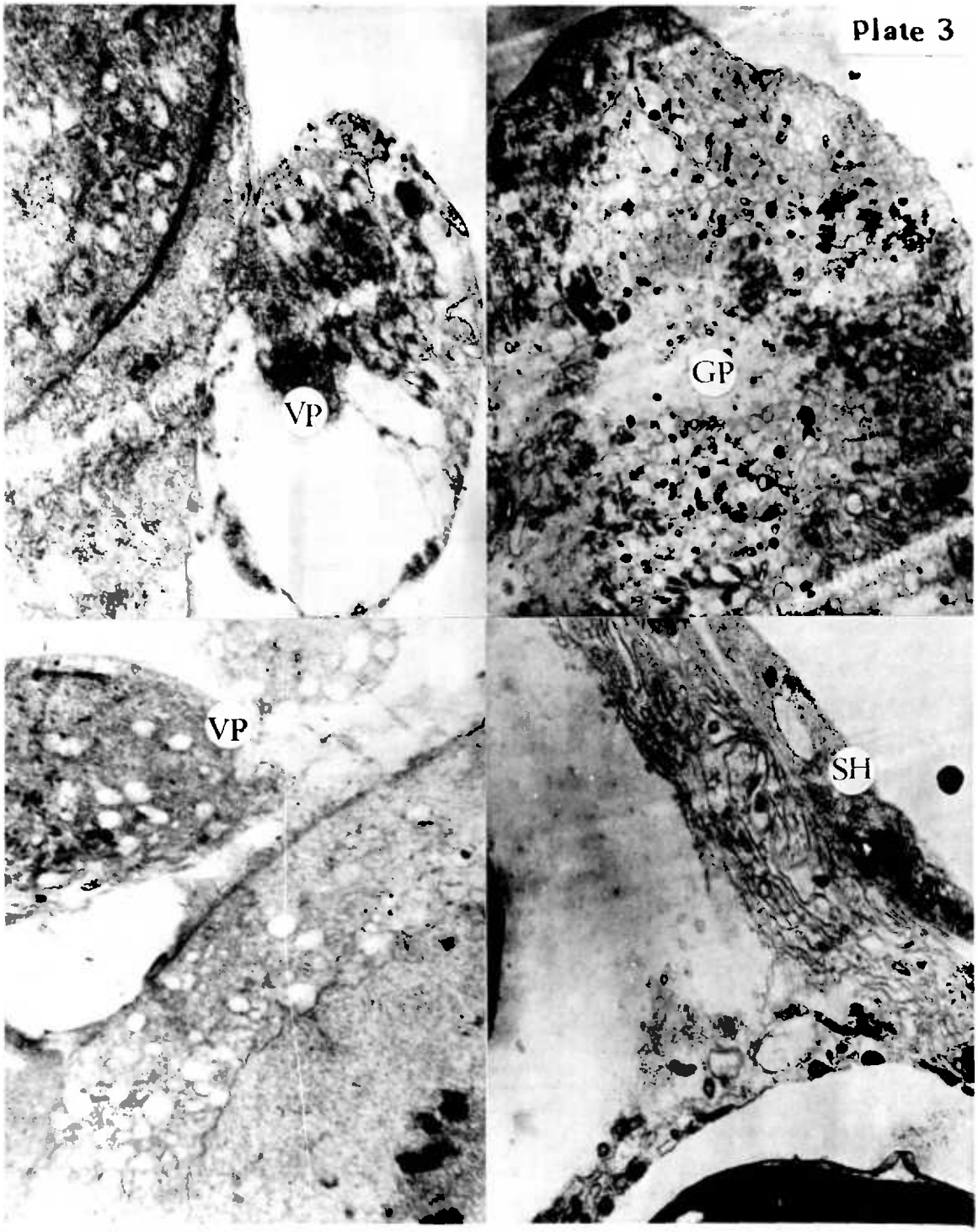
SH. Spindle-shaped Haemocytes flattened externally on the capsule surface.

VP. Vaculated plasmatocyte, attached to the capsule.

GP. Granulated plasmatocyte.

Top left	(X 10792)
Top right	(X 15390)
Bottom left	(X 9120)
Bottom right	(X 9120)

Plate 3



capsule as third stage larvae. In the present study, capsules have been examined from different life stages of the host, at the time when these capsules exhibit different distinct developmental stages.

Capsules from pupae contained the developing adult female nematodes. These capsules consisted of loosely packed vesicles and vacuoles (Plate 4), the vesicles differed in size and shape; they were either spherical or elongated and the size varied between 0.2 - 0.4 µm in diameter, they were bound with a double membrane. These vesicles presumably formed when the haemocytes firstly made contact with the parasites, sending out numerous fine cytoplasmic extrusions from their surfaces resulting in the formation of these vesicular elements within the capsule. The capsule wall varied in thickness, ranging between 3 - 8 µm and was related to the parasite density per host and number of nematodes in each capsule. The capsule contained a variety of cytoplasmic structures which were originally haemocyte inclusions (Plates 4 and 5). The membranes surrounding the cytoplasmic structures were the plasma membrane of the haemocytes. These membranes formed the complex meshwork of tubules throughout the cytoplasm, which is the endoplasmic reticulum. When the cell membranes breakdown and the protoplast of these cells merged together,

PLATE 4

TRANSVERSE SECTION OF THE CAPSULE FROM PUPAL HOST

Key

Er	Endoplasmic reticulum
dg	dense granules (Probably mucopoly-saccharide)
rb	ribosome
ne	nematode
gc	golgi complex
db	dense bodies
m	mitochondria
mt	microtubules
va	vacuoles
ve	vesicle

(X 15390) Top

(X 23940) Bottom

(X 50160) inset

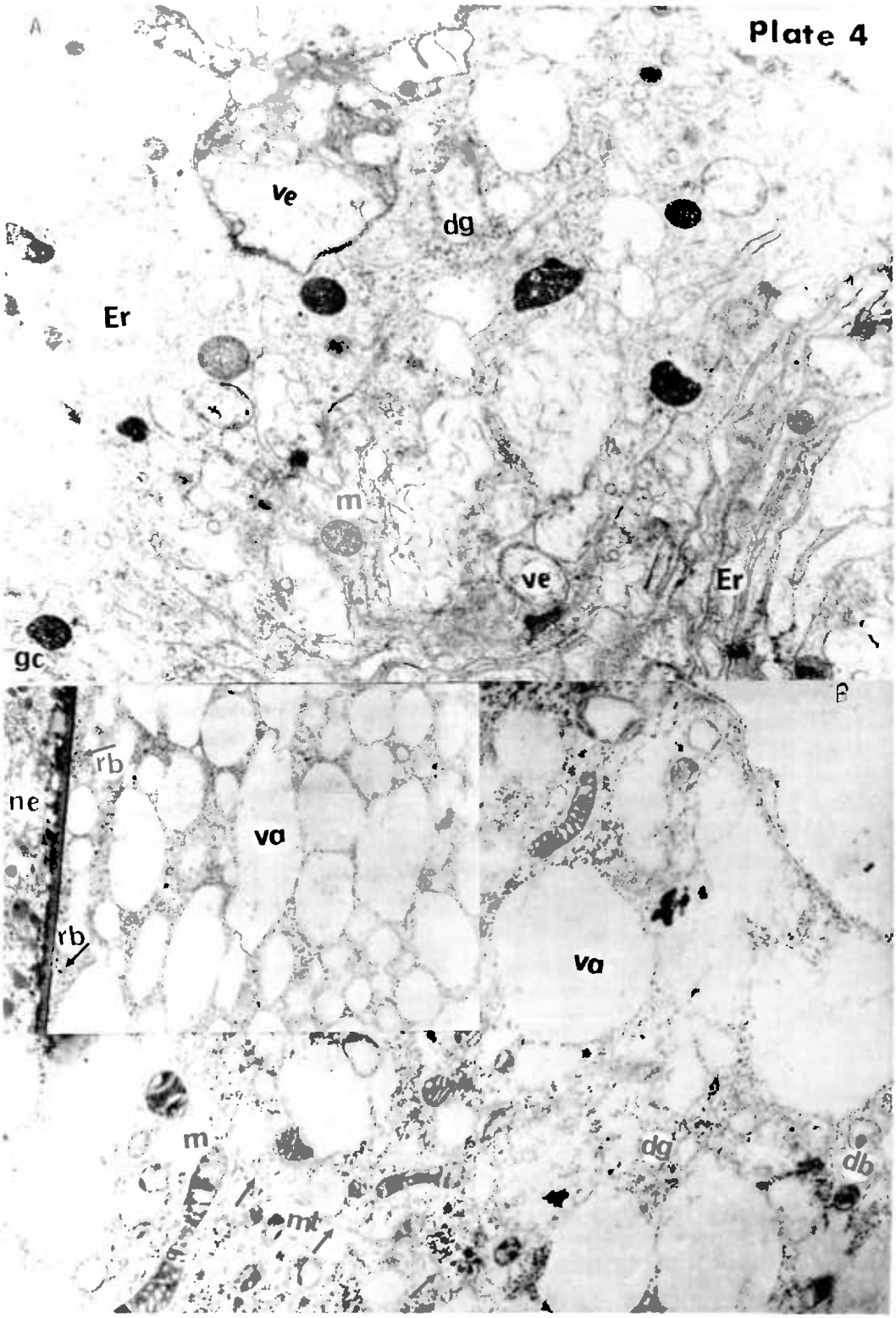


Plate 4
A
ve
dg
Er
m
ve
Er
gc
rb
ne
va
rb
va
B
m
mt
dg
db

PLATE 5

CAPSULE FROM PUPAL HOST SHOWING HOW THE HAEMOCYTES LYSED AND MERGED THEIR CONTENTS TO THE CAPSULE'S CYTOPLASM.

Key

cm	Haemocyte loosing its cell membrane at the point of contact and merging its cytoplasmic content to the capsule (arrow)
db	dense bodies
gc	golgi complex
m	mitochondria
nu	nucleus
Tr	tracheoles

(X 12141) Top (right or left)

(X 4788) Bottom

Plate 5

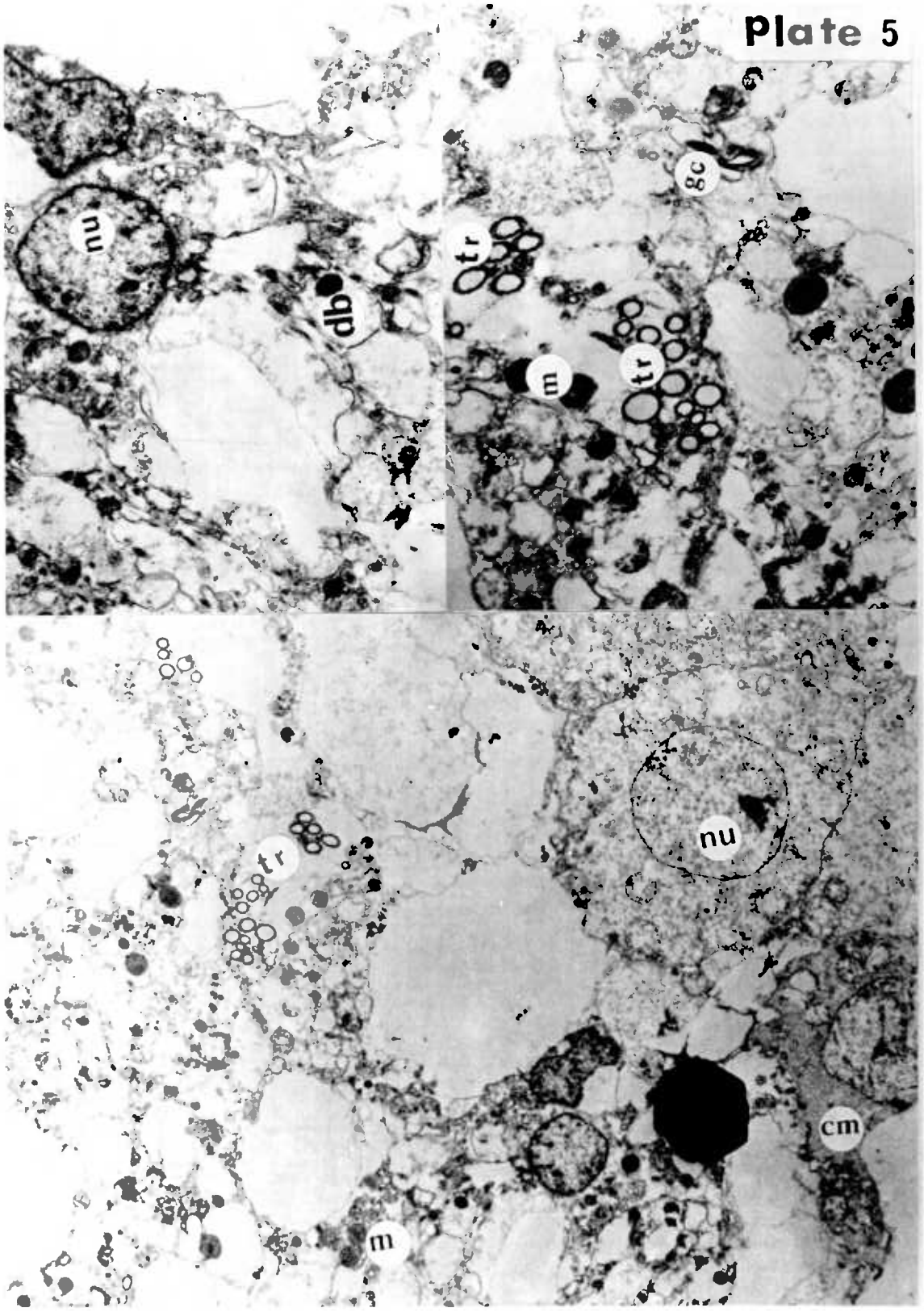


PLATE 6

SECTION OF THE CAPSULE FROM PUPAL HOST, SHOWING ITS DIFFERENT STRUCTURE AND ORGANELLES.

Key

gc	golgi complex
mt	microtubules
m	mitochondria
ser	smooth endoplasmic reticulum
rer	rough endoplasmic reticulum
va	vacuoles
ve	vesicles

(X 17100) A

(X 13680) B

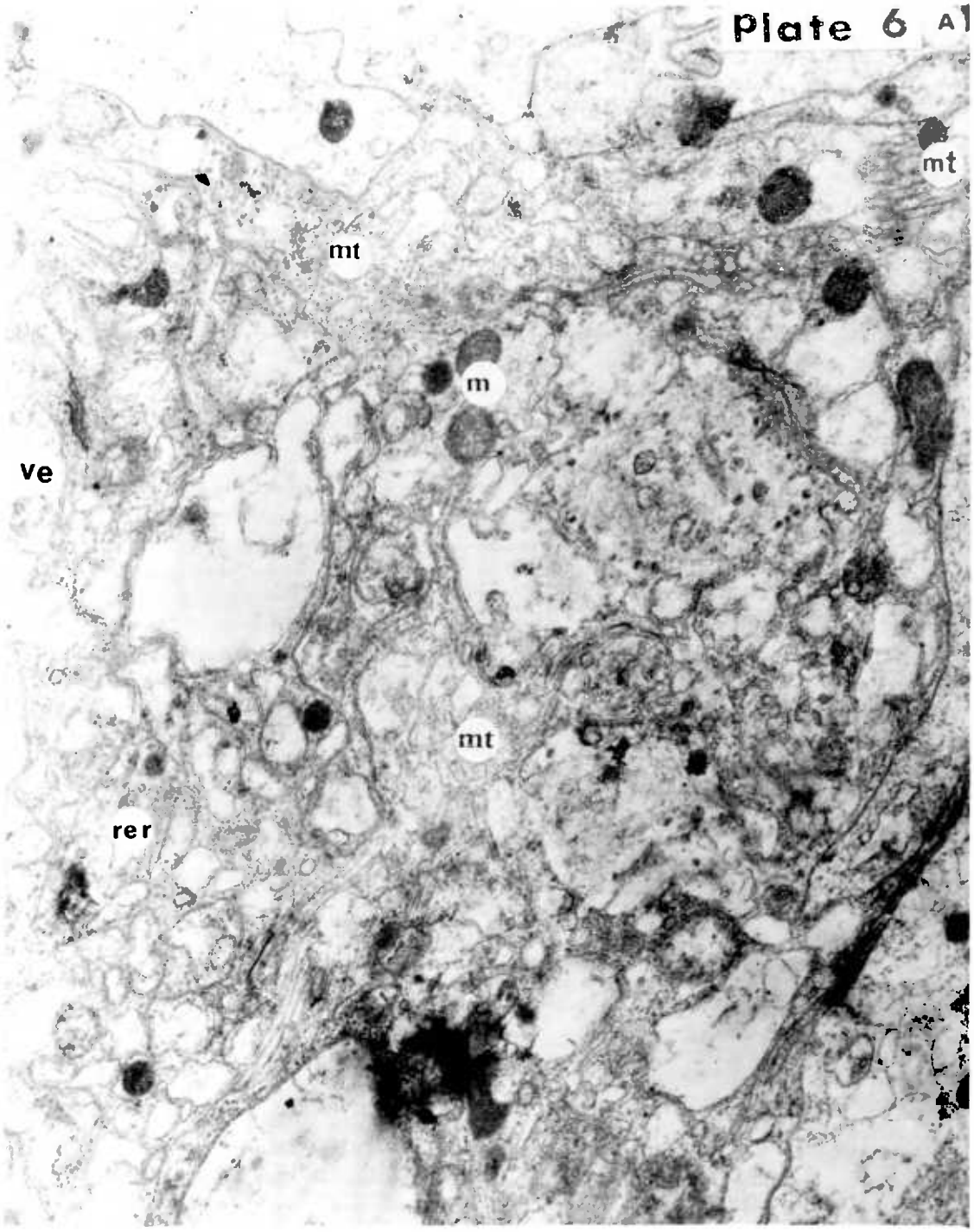


Plate 6 A1
mt
mt
m
ve
mt
rer

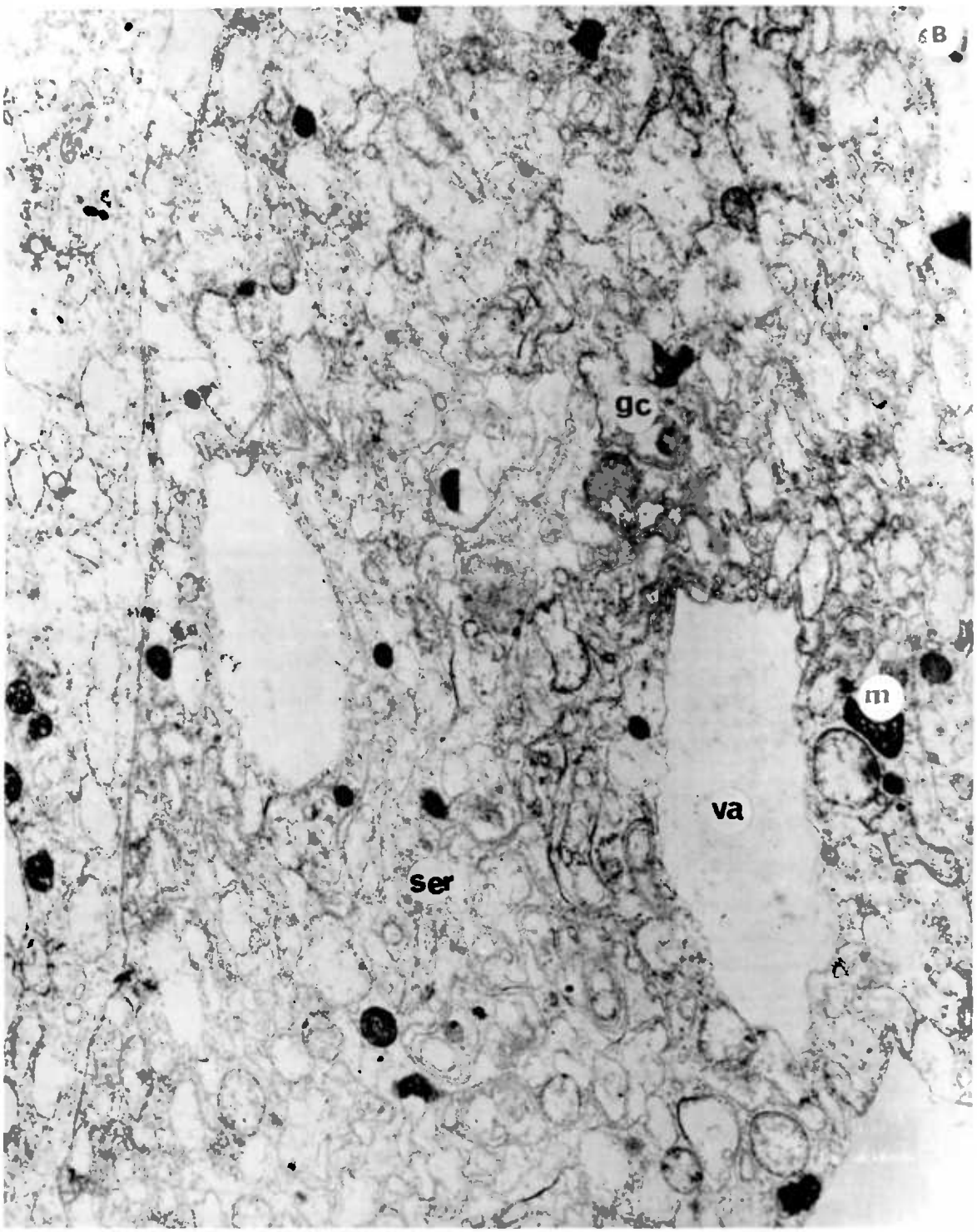

6B
gc
m
va
ser

PLATE 7

SECTION OF THE CAPSULE FROM PUPAL HOST, SHOWING (M) MITOCHONDRIA, (MT) MICROTUBULES, (TR) TRACHEOLES AND ENDOPLASMIC RETICULUM (ER)

Key

m	mitochondria
mt	microtubules
tr	tracheoles
er	endoplasmic reticulum
rb	ribosomes

(X 84080)

Plate 7

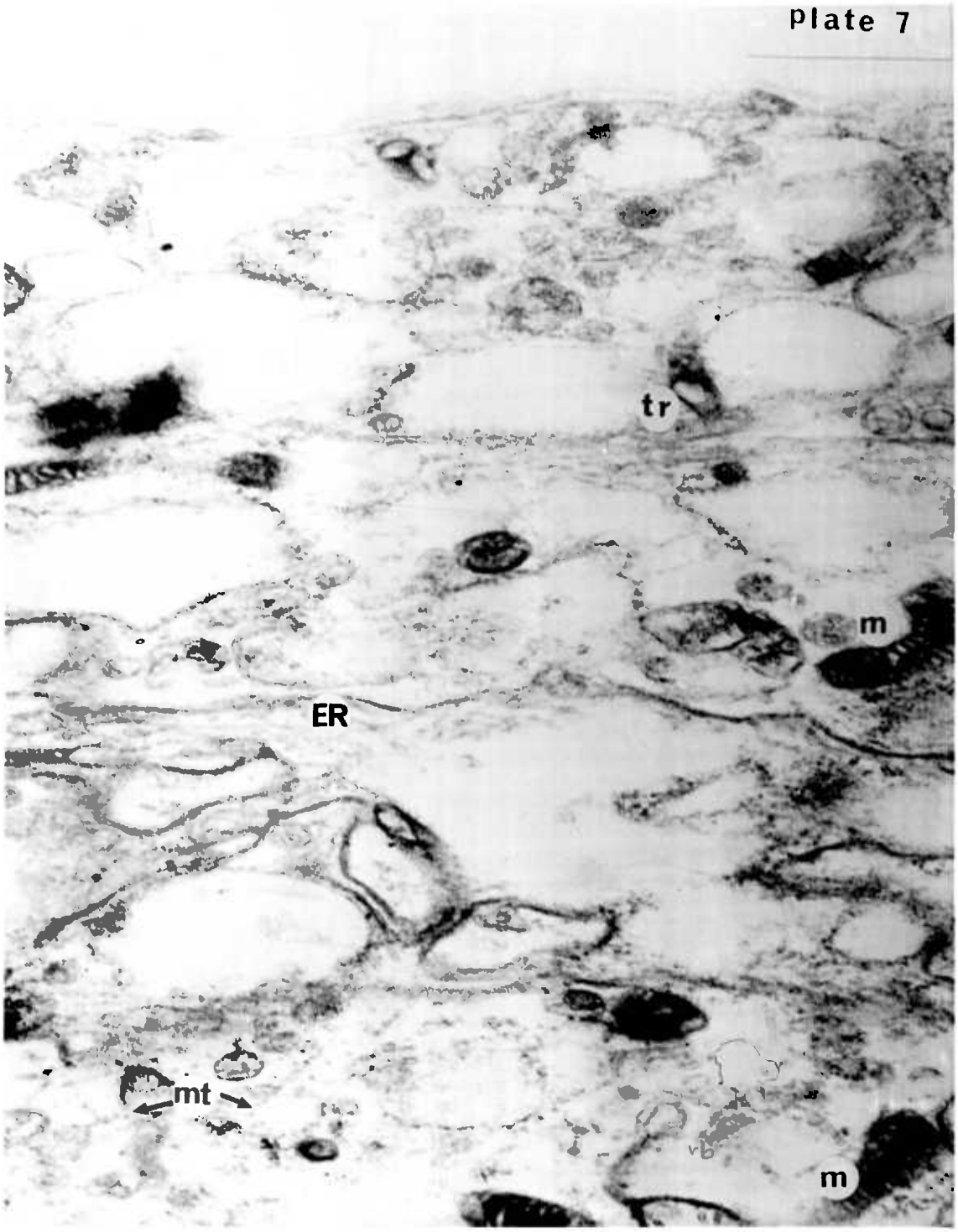


PLATE 8

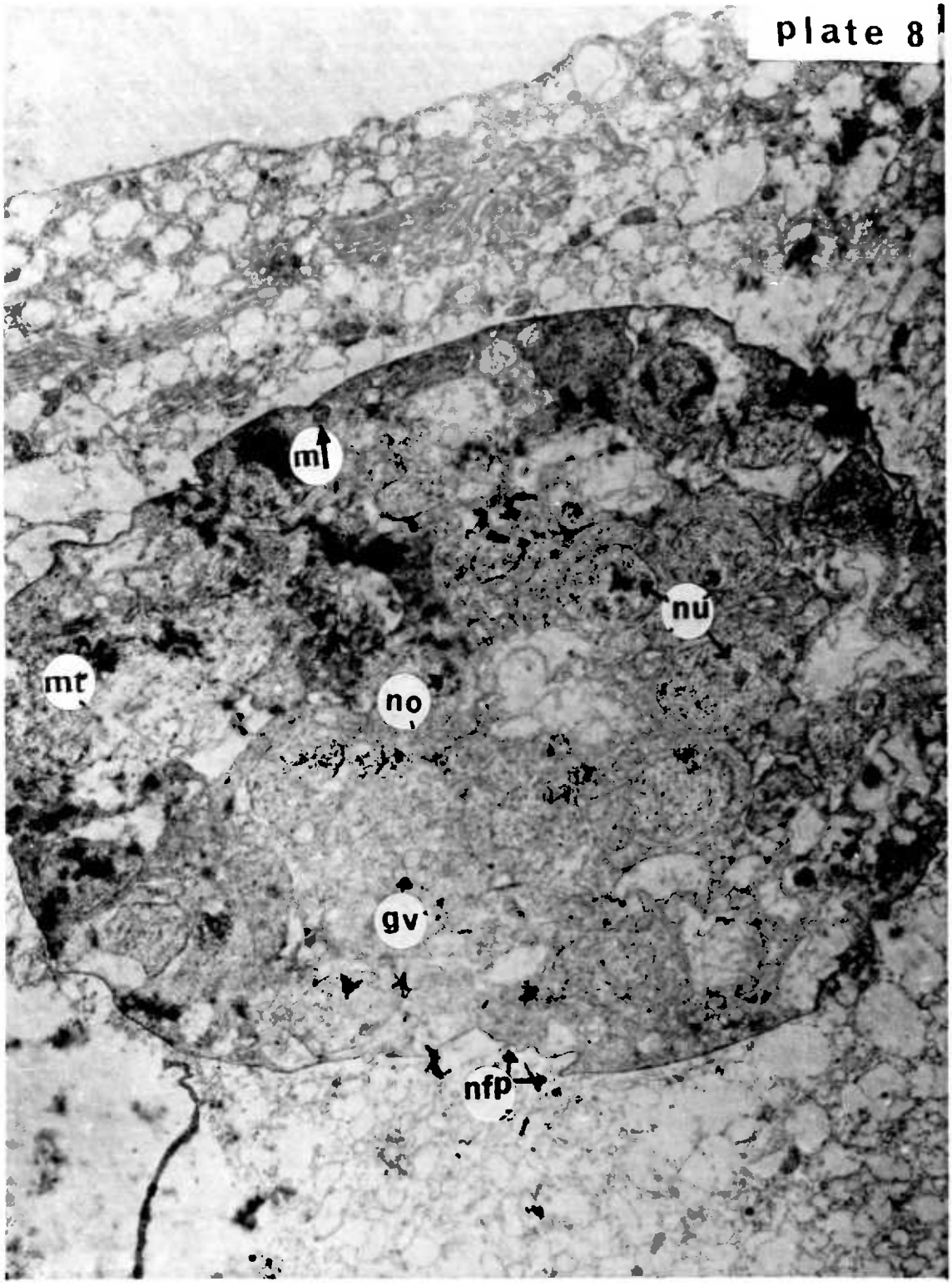
MULTINUCLEATE PLASMATOCYTES REMAINING INTACT WITHIN THE CAPSULE TISSUE FROM THE PUPAL HOST

Key

nfp	numerous fine pseudopodia
nu	nucleus
no	nucleolus
gv	golgi vesicle
m	mitochondria
mt	microtubules

(X 9120)

plate 8



forming a syncytium surrounding the nematode (Plate 5). Numerous microtubules were associated in this capsule (Plate 6 A), varied mitochondria, spherical or rod-shaped with irregular cristae (Plates 4, B, 6, B,). Very fine dense bodies, possibly ribosomes, were sparsely scattered around the nucleus and were abundant between the vesicles and the nematodes (Plate 4). Small lysosome-like dense bodies which varied between 0.15 - 0.2 µm in diameter were observed (Plate 6 A, B). Intact multinucleate haemocytes with 8 - 10 nuclei were found with very fine pseudopodia (Plate 8), the multinucleate cells were possibly formed by mitotic division. Haemocytes with vacuolated cytoplasm were found attached externally to the capsule, these cells were the plasmatocytes of the pupal host which were vacuolated (Plate 3). It was impossible to differentiate the capsule tissue into various regions or zones because it appeared as a homogenous layer, containing all these organelles in Plate 4 and 5. Fine tracheoles started to form in this capsule but not in abundance (Plate 5).

The nuclei

The capsule from the pupal host contained irregular amoeboid shaped nuclei, nuclear pores were not very often (Plates 9 A, B and C) seen. These nuclei had a normal

PLATE 9

SECTION OF THE CAPSULE FROM HOST PUPA, SHOWING THE AMOEBOID SHAPED NUCLEI AND NUCLEAR PORES.

Key

dgb.	dense granular bodies surrounding the nucleus, the vesicles and the nematode
ne	nematode
no	nucleolus
nu	nucleus
np	nuclear pore (shown on plate 6 B as du)
rb	ribosomes
va	vacuoles

A (X 6384)

B (X 25080)

C (X 6384)

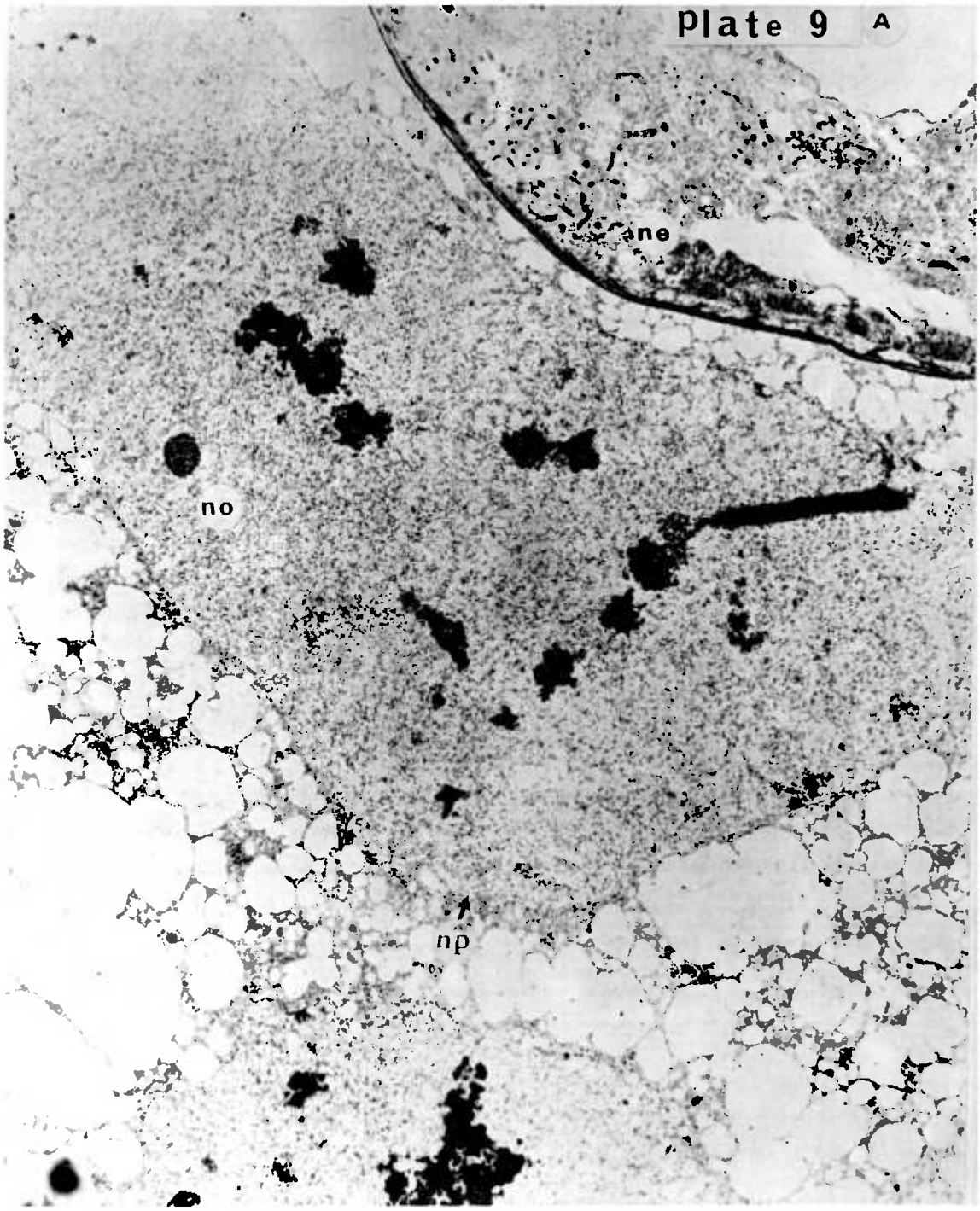


Plate 9 A
ne
no
np


va
rb
dgb
du
ch

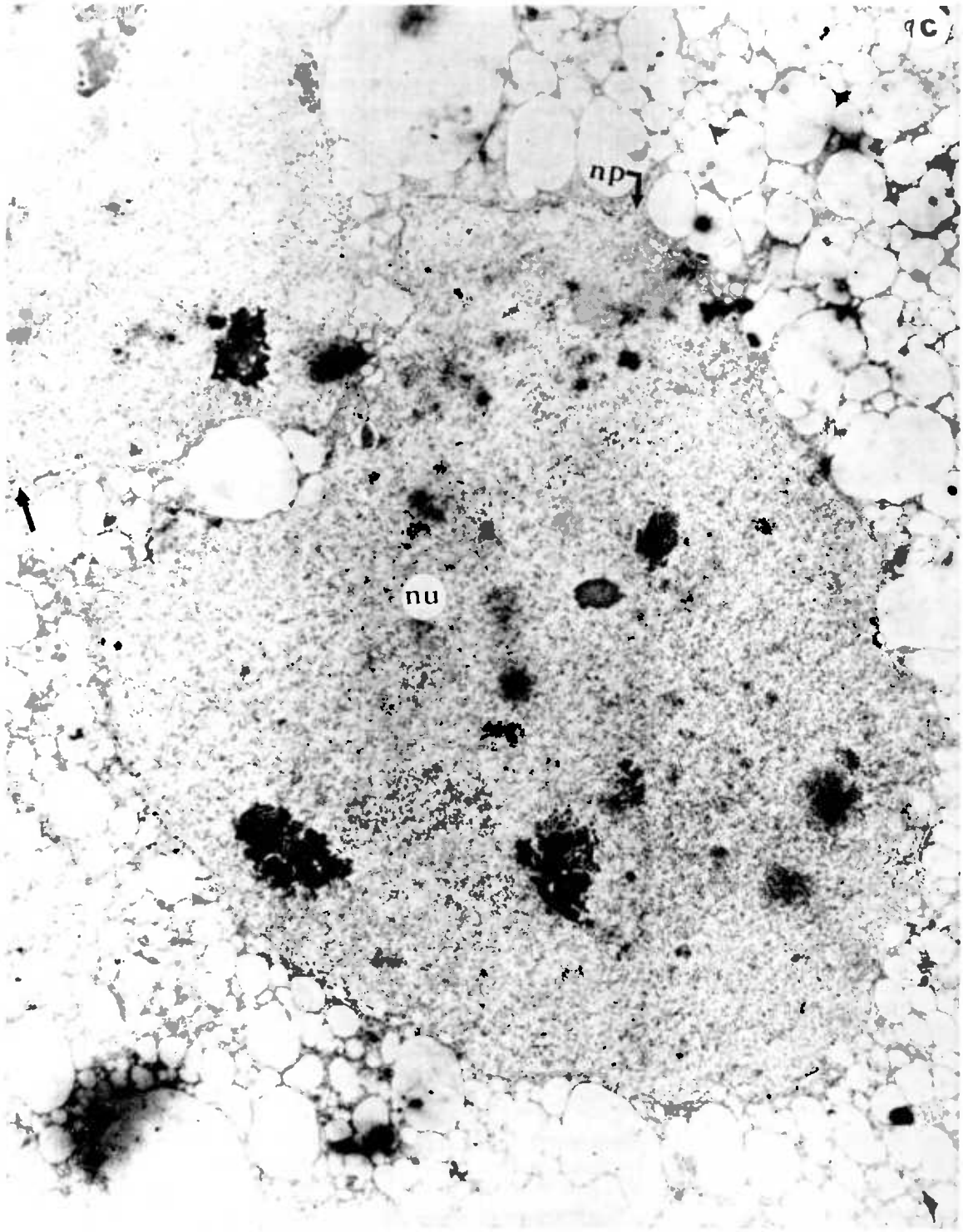

np
nu

PLATE 10

ELECTRON MICROGRAPH FOR THE CAPSULE FROM A PUPA, SHOWING THE INITIAL CHANGES THAT OCCUR IN THE NUCLEUS AND NUCLEAR MEMBRANE. 3 TO 4 NUCLEAR MEMBRANES ARE DISTINCT CONTAINING SMALL DENSE BODIES SIMILAR IN APPEARANCE TO THOSE IN THE CYTOPLASM.

Key

ch	chromatin material scattered towards the nuclear membrane
db	dense bodies within the pocketings
m	mitochondria
mt	microtubules
mp	nuclear membrane pocketings
nm	4 nuclear membranes
nu	nucleus
va	vacuoles

(X 171000)

plate 10

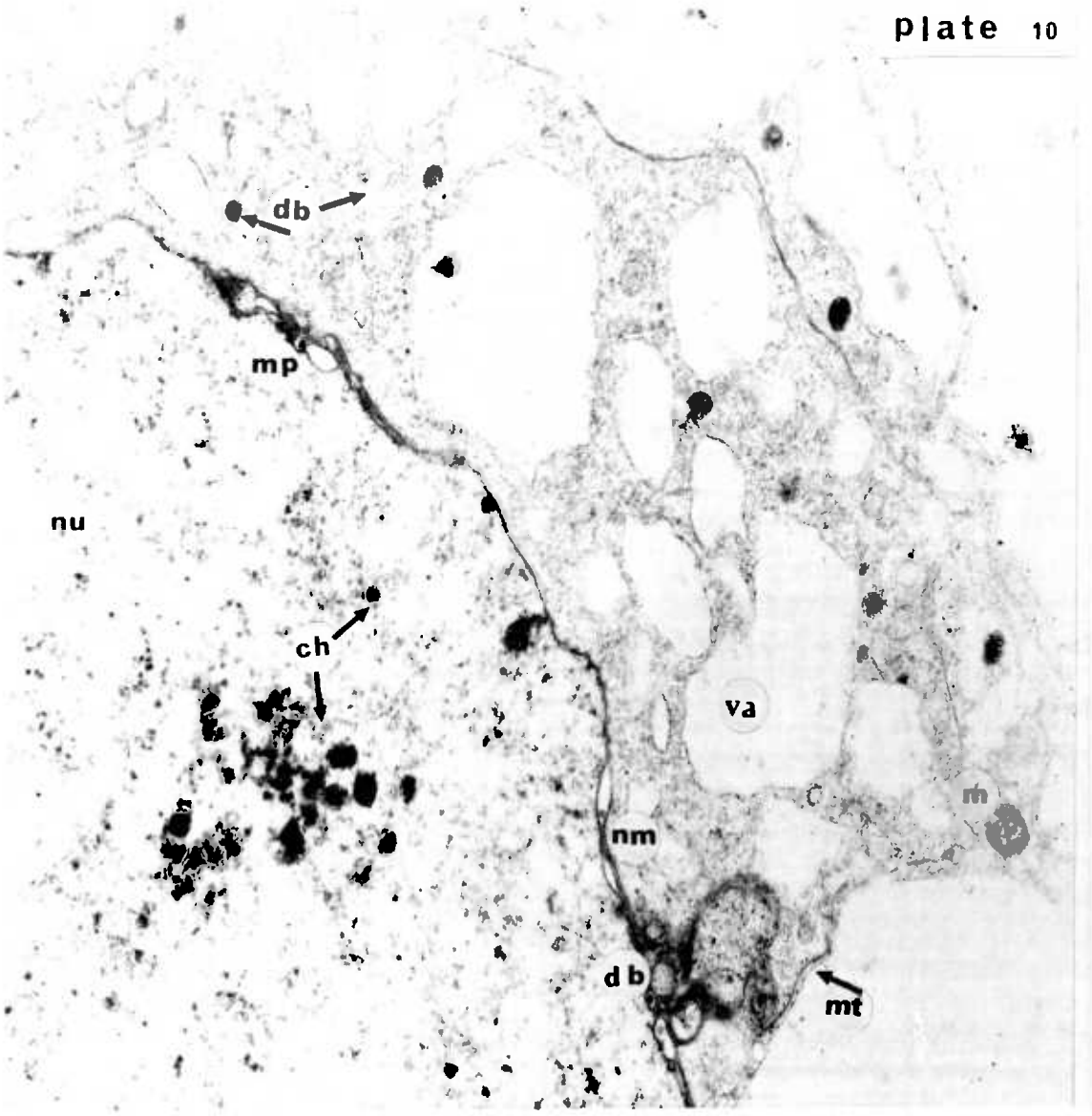


PLATE 11

CAPSULE WALL FROM YOUNG ADULT HOST BEETLE SHOWING THE VARIATIONS IN THE STRUCTURE OF THE WALL AND THE REGIONS THAT APPEAR IN THE CAPSULE.

Key

1	outer region
2	middle region (contains flattened and compressed haemocytes)
3	inner region
db	dense bodies (probably lysosomes)
gb	golgi bodies
Tr	trachea
tr	tracheoles
er	endoplasmic reticulum
fc	flattened cell
Top	Entire section through the capsule wall (X 25080)
Bottom right	Part of the outer region of capsule wall (X 2508)
Bottom left	Middle and outer region of the capsule wall (X 2508)

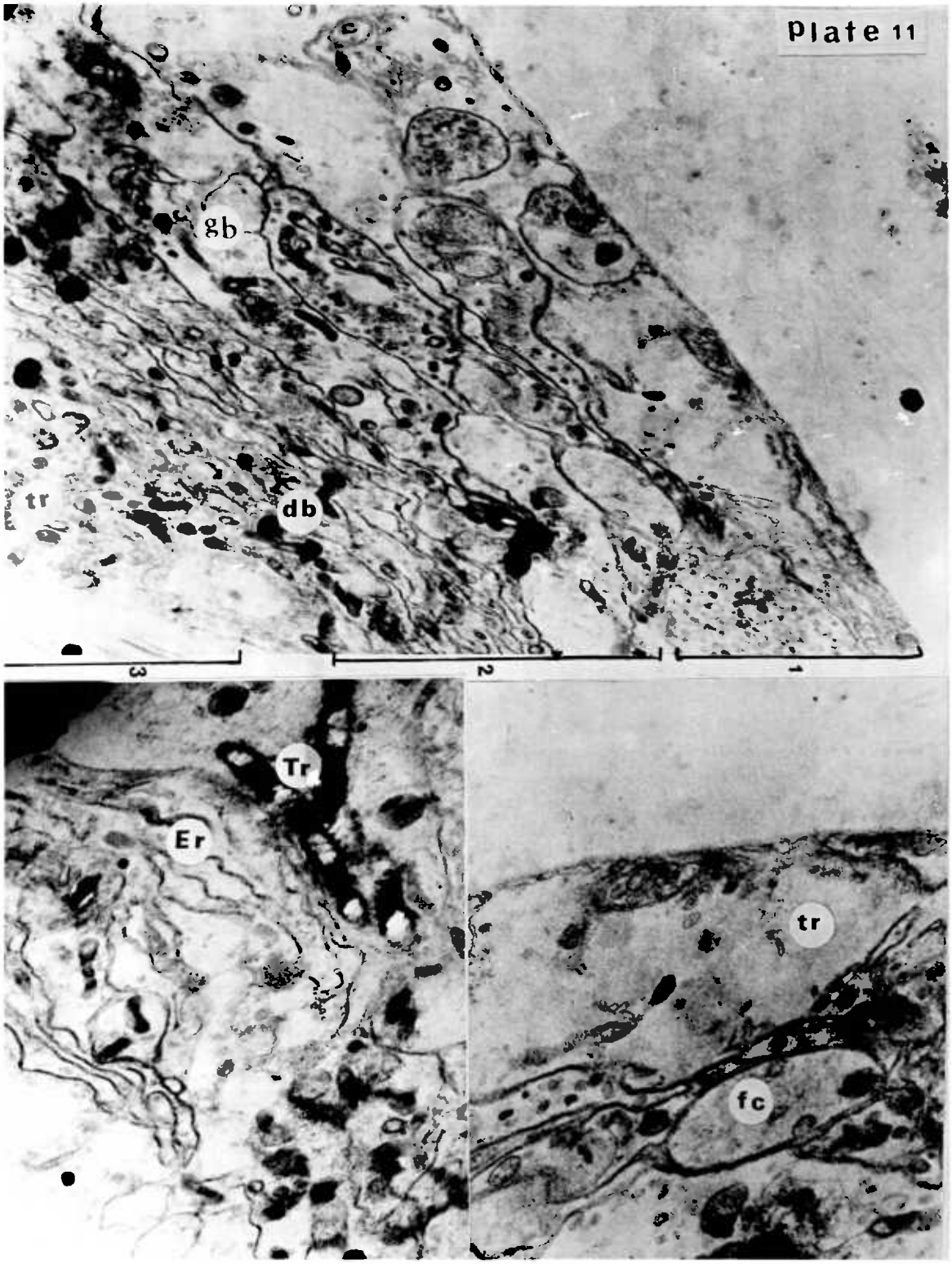


Plate 11
gb
tr
db
3
2
1
Tr
Er
tr
fc

PLATE 12

THE ENTIRE CAPSULE WALL FROM A YOUNG ADULT BEETLE SHOWING THE DIFFERENT REGIONS

Key

db	dense bodies (lysosomes)
gb	golgi bodies
gh	granular haemocyte
mt	microtubules
tr	tracheoles
rb	ribosomes
or	outer region
mr	middle region
ir	inner region
ocw	outer capsule wall
icw	inner capsule wall

(X 50160)

Plate 12

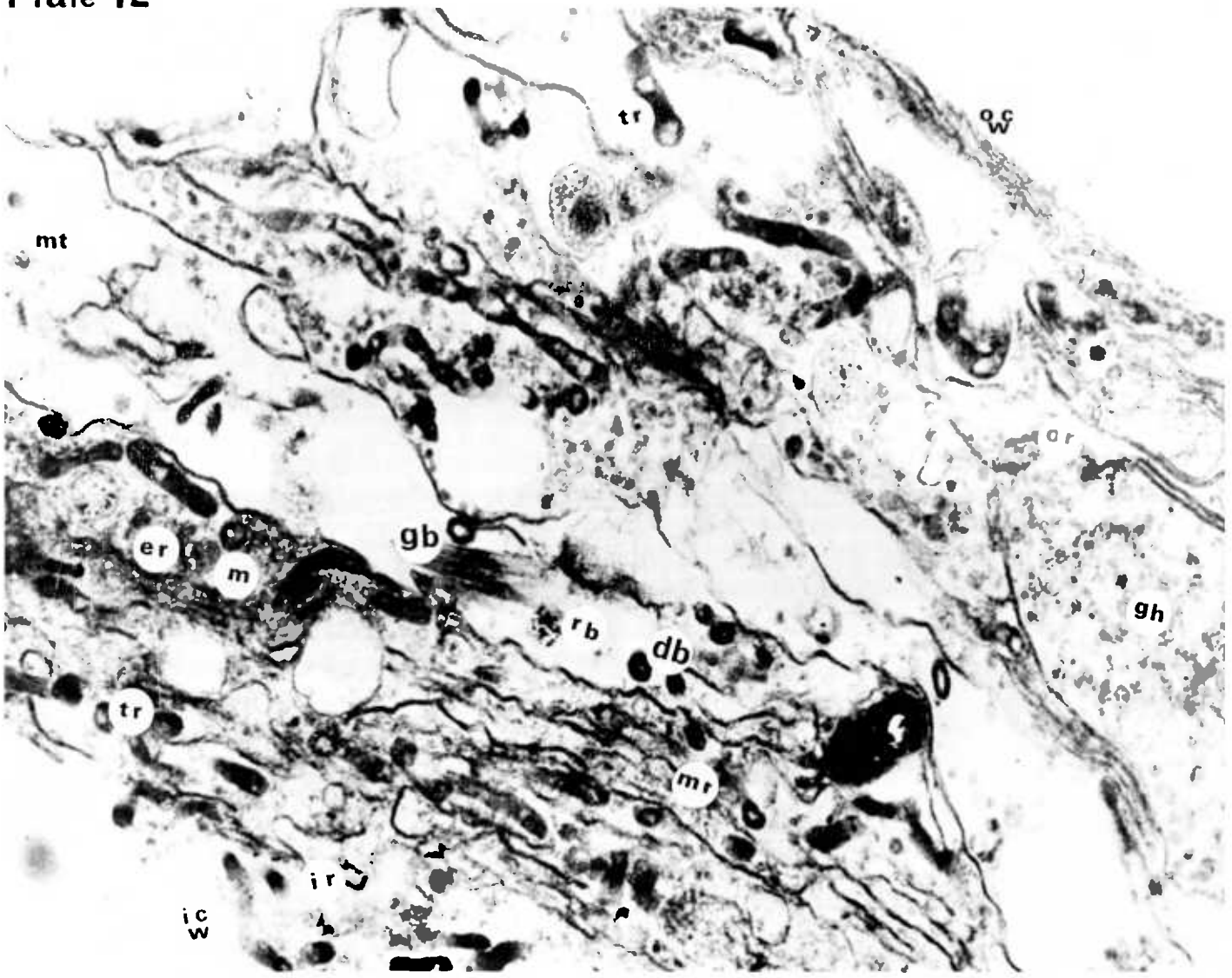


PLATE 13

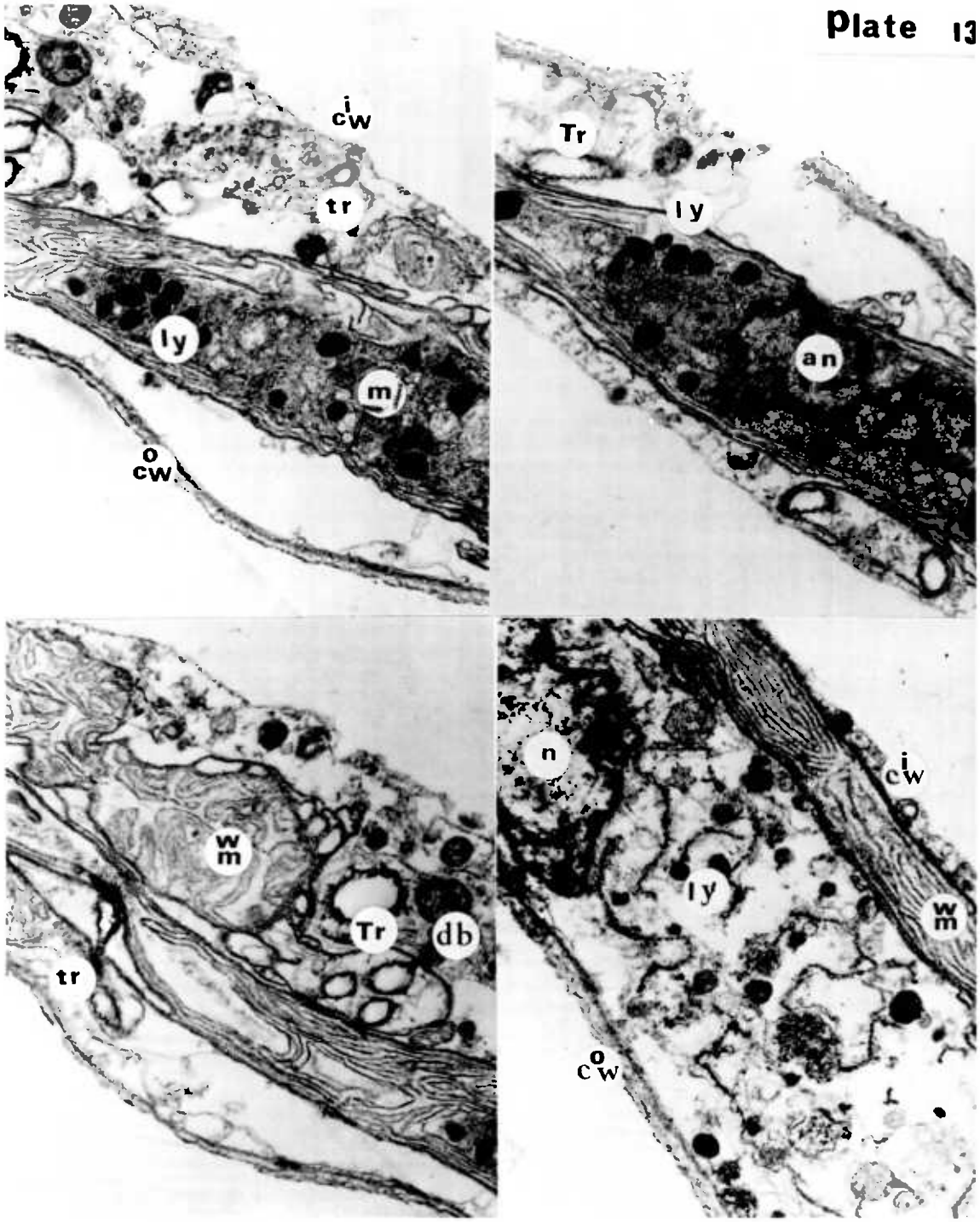
FOUR SECTIONS OF DIFFERENT CAPSULES FROM OLD ADULT BEETLES, SHOWING THE OLD CAPSULE WALL STRUCTURE WHERE NO REGIONS CAN BE DIFFERENTIATED.

Key

an	amoeboid shaped nucleus
db	dense bodies
ly	lysosomes
ocw	outer capsule wall
icw	inner capsule wall
m	mitochondria
n	nucleus
Tr	trachea
tr	tracheoles
wm	whorls of membranes

(X 12540)

Plate 13



double nuclear membrane, but later in a capsule at a different stage of development, it contained nuclei in different stages of physiological activities. Nuclei in a capsule of pupal hosts could be considered normal but highly specialized when they started to become bigger and amoeboid in shape. The breakdown of the cell structure, the disintegration of the cell membranes, permits the gathering of the nuclei. This was followed by nuclear extrusion of the dense bodies which were then found free in the cytoplasm. These dense bodies also disintegrated as the nematode developed. There was, however, a continuous replacement of nuclei and cytoplasm from more haemocytes adding to the capsule externally when the reaction continued and the parasites were developing.

Pocketing of the nuclear membrane was either entirely absent in recently formed capsules (Plate 9 A, B, C) or was detected in capsules of beetles nearing the completion of pupation (Plate 10). When the mechanism of nucleocytoplasmic interaction began, the swelling of the nuclear membrane started (Plate 10), a triple nuclear membrane appeared and then dense bodies on both sides of the membrane were visible. In capsules from the adult host the nuclear membrane was found to be undulatory and frequently to protrude into the cytoplasm (Plates 14 (A, B), 15 A, 15 B, and 20), in the form of small pocketings contained

PLATE 14

ELECTRON MICROGRAPH SHOWING THE ALTERATION IN THE NUCLEAR STRUCTURE OF CELLULAR CAPSULES FROM ADULT BEETLES. POCKETINGS AND THE FRAGMENTATION OF THE CHROMATIN MATERIAL THAT MOVES TOWARDS THE NUCLEAR MEMBRANE IN FORM OF DENSE BODIES.

Key

ch	chromatin material
db	dense bodies (arrows)
er	endoplasmic reticulum
gc	golgi complex
m	mitochondria
mt	microtubules
nu	nucleus
mp	pocketing of the nuclear membrane

(X 7600) Top

(X 13680) Bottom

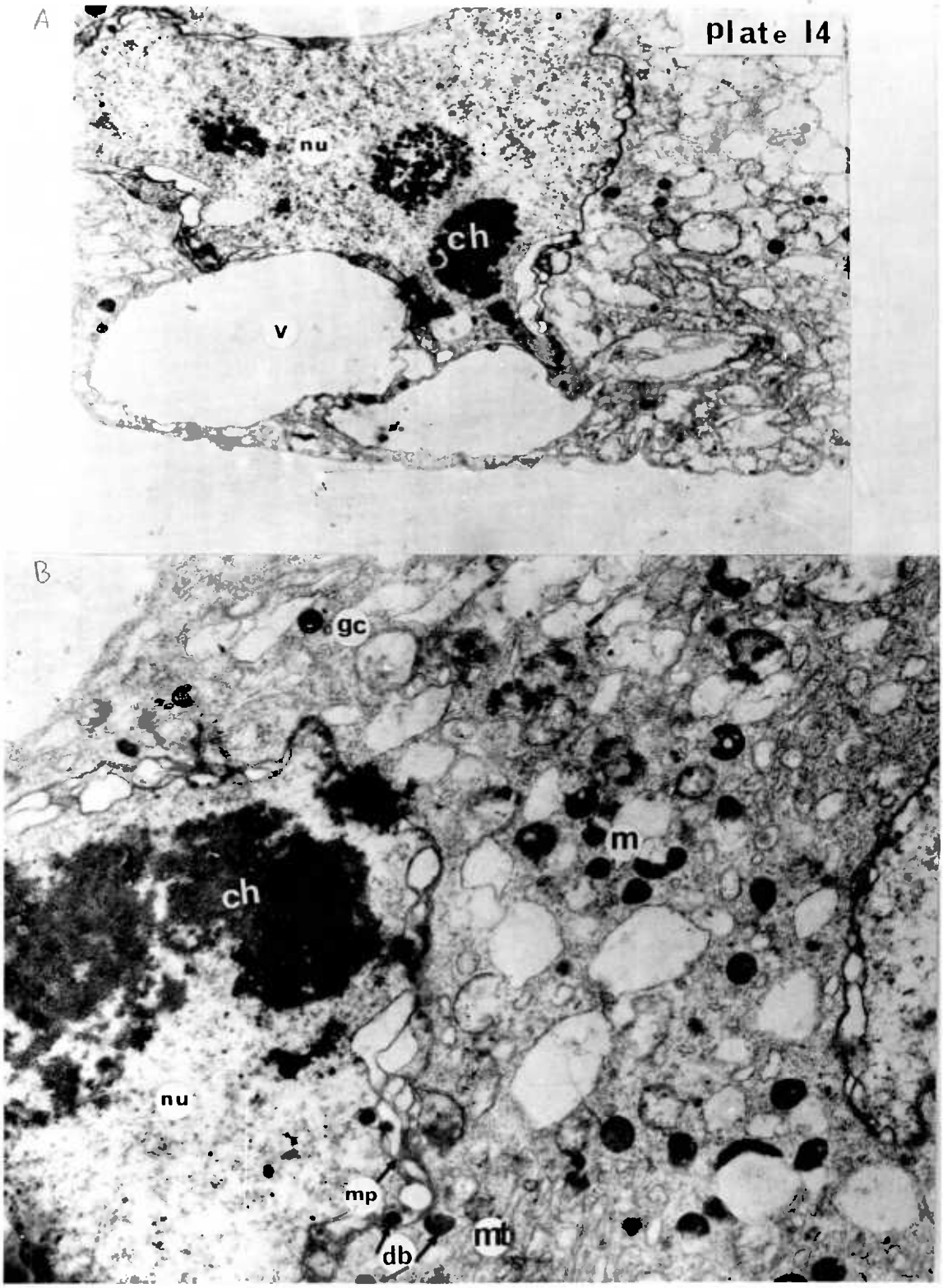

A
plate 14
nu
ch
v
B
gc
m
ch
nu
mp
db
mb

PLATE 15

SECTIONS OF CAPSULE FROM ADULT BEETLES, SHOWING THE CHANGES THAT OCCURRED TO THE NUCLEAR MEMBRANE

Key

ch	chromatin
er	endoplasmic reticulum
gv	golgi vesicles
m	mitochondria
mt	microtubules
mp	membrane pocketings
nu	nucleus

A (X 11725.7) top
(X 10260) middle
(X 4788) bottom
B (X 1172)

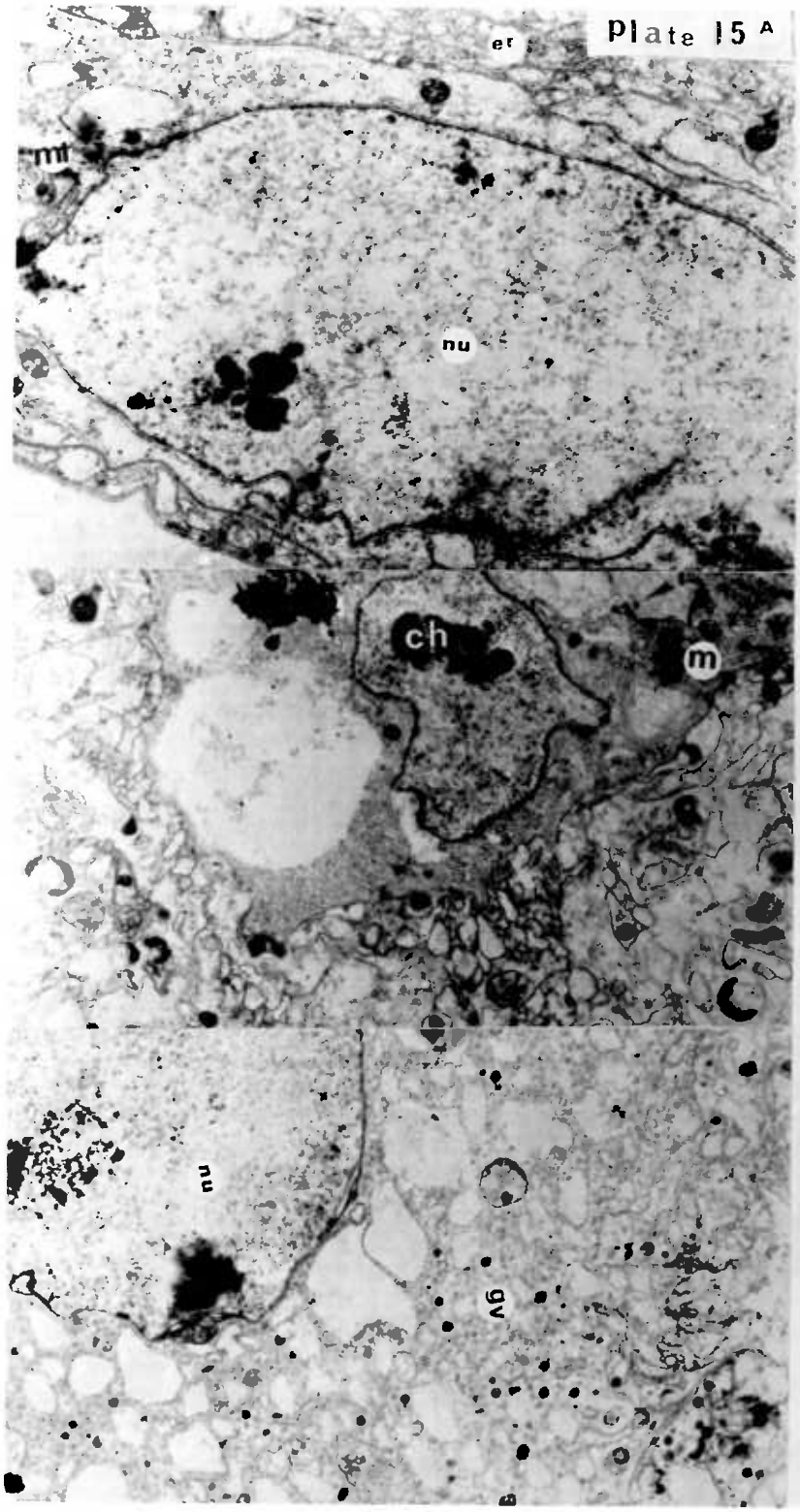
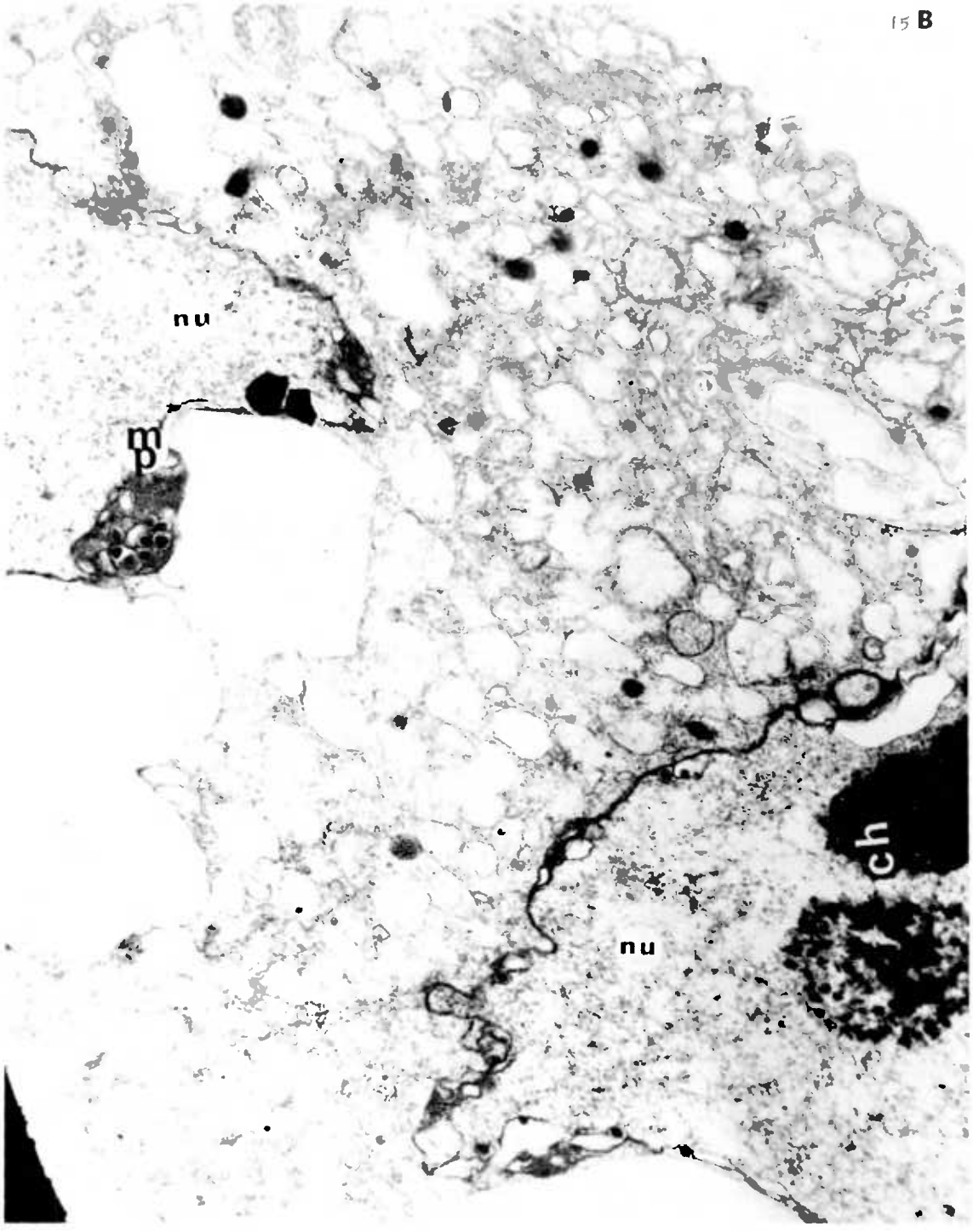


Plate 15 A
er
mt
nu
ch
m
nu
gv

15 B



these dense bodies. The nuclear membranes became indistinct in the later stages of the capsule development, in old adult beetles (Plate 21). The nuclei degenerate and their contents pass through the nuclear membranes or nuclear pores into the cytoplasm in the form of small electron dense bodies. Deep folding of the nuclear membrane was often present in big nuclei (Plate 16) in a capsule from young adults when the nematodes are growing rapidly at that time, possibly these folds increase the nuclear surfaces and may have contributed to the passage of the nuclear substances to the cytoplasm. Plates 13 and 16 showed these nuclei with their deep foldings and the stained chromatin material scattered along the inner boundary of the nuclear membrane. Particles of similar size and density on both sides of the nuclear membrane were observed, even in contact with it, (Plates 14 B, 15 B) possibly they were in the process of passing through when they fixed. These pocketings were possibly the characteristic features of the capsule from young adult beetles and they possibly represent an early stage in the degeneration of the nuclei. The growing nematodes within the capsules received soluble products from the cytoplasm of the capsule, which comprised the cytoplasm liberated by the breakdown of the cell membranes and certain material which

was extruded from the nuclei, through the nuclear membrane. In the developmental stages the nuclear membranes are actively functioning organelles (Plates 14 (A,B), 15 A, 15 B, and 16). Nuclear extrusions in a form of dense bodies is possibly the DNA of the nuclei, they were similar to those in the cytoplasm.

— Capsules from young adult hosts contained the mature adult parasitic females and her eggs and larvae. These capsules had a recogniseable cellular structure, the haemocytes still being attached to the outer surface. These cells have normal structure (Plate 3).

Three regions can be distinguished in this capsule (Plate 11). The haemocytes that attached themselves to the capsule externally lost their cell membrane at the point of contact and merged their cytoplasmic contents.

Trachea were often found to be attached externally to the capsule (Plates 22 and 23) and small tracheoles were embedded in its tissue (Plates 11, 12, 13, 22 and 23) The tracheation of the capsule started soon after infection of the pupae occurred, but became more abundant as the capsule and the nematode developed. It is possible that tracheation is linked to an oxygen demand. Heavier tracheation was found in capsules from highly infected host, although the majority of the capsules were found ramified

with tracheae, but it was lighter in the capsule from hosts with one to three parasites. The presence of these parasites presumably affected the respiration of their hosts.

The outer region of the capsule is semi-opaque and comprises relatively rounded or spindle shaped cells, some of them slightly flattened or in the process of flattening. The inner cells become flattened and are compressed, the flattening occurs in parallel position to the parasites. In the section this region appears to compose of overlapping cells (Plates 11 and 12).

In the middle region the cells are extremely compressed and flattened (Plate 11). In this region the cells piled upon each other forming a compact zone (Plates 11 and 12). These flattened cells contained numerous dense granules, presumably lysosomes 0.39 - 0.45 μm in diameter. No sign of necrosis was found in this region. Mitochondria ranged between 0.3 - 0.6 μm and sometimes they were as small as 0.1 μm in diameter (Plates 13, 16 and 17). Microtubules and golgi bodies were found in this region (Plate 12) while other electron dense small bodies, presumably ribosomes were present (Plate 12). Tracheoles ranging between 0.39 - 0.6 μm in diameter and trachea between 0.6 - 3.1 μm in length were abundant (Plate 11,

PLATE 16

ELECTRON MICROGRAPH SHOWING CAPSULE TISSUE FROM YOUNG ADULT HOST BEETLE PACKED WITH NUCLEI, MITOCHONDRIA AND OTHER ORGANELLES

Key

b	membrane bounded bodies
m	mitochondria
ne	nematode
no	nucleoles
nu	nucleus
tr	tracheoles

(X 47880) top

(X 28728) bottom

Plate 16

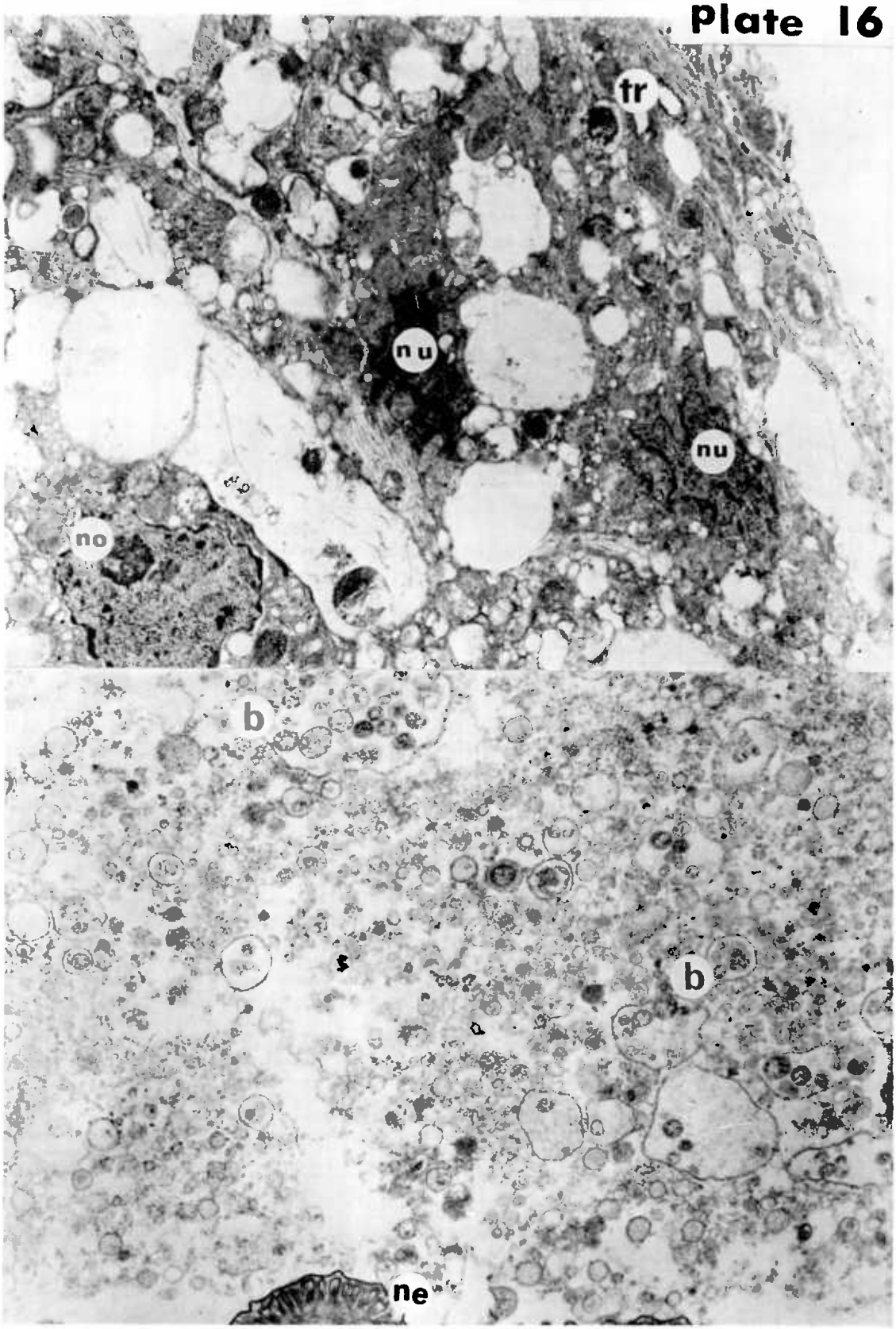


PLATE 17

ELECTRON MICROGRAPH SHOWING CAPSULE TISSUE FROM YOUNG ADULT HOST BEETLE WITH DIFFERENT SHAPES OF MITOCHONDRIA, AND TRACHEOLES.

(X 27360)

Key

m	mitochondria
tr	tracheoles

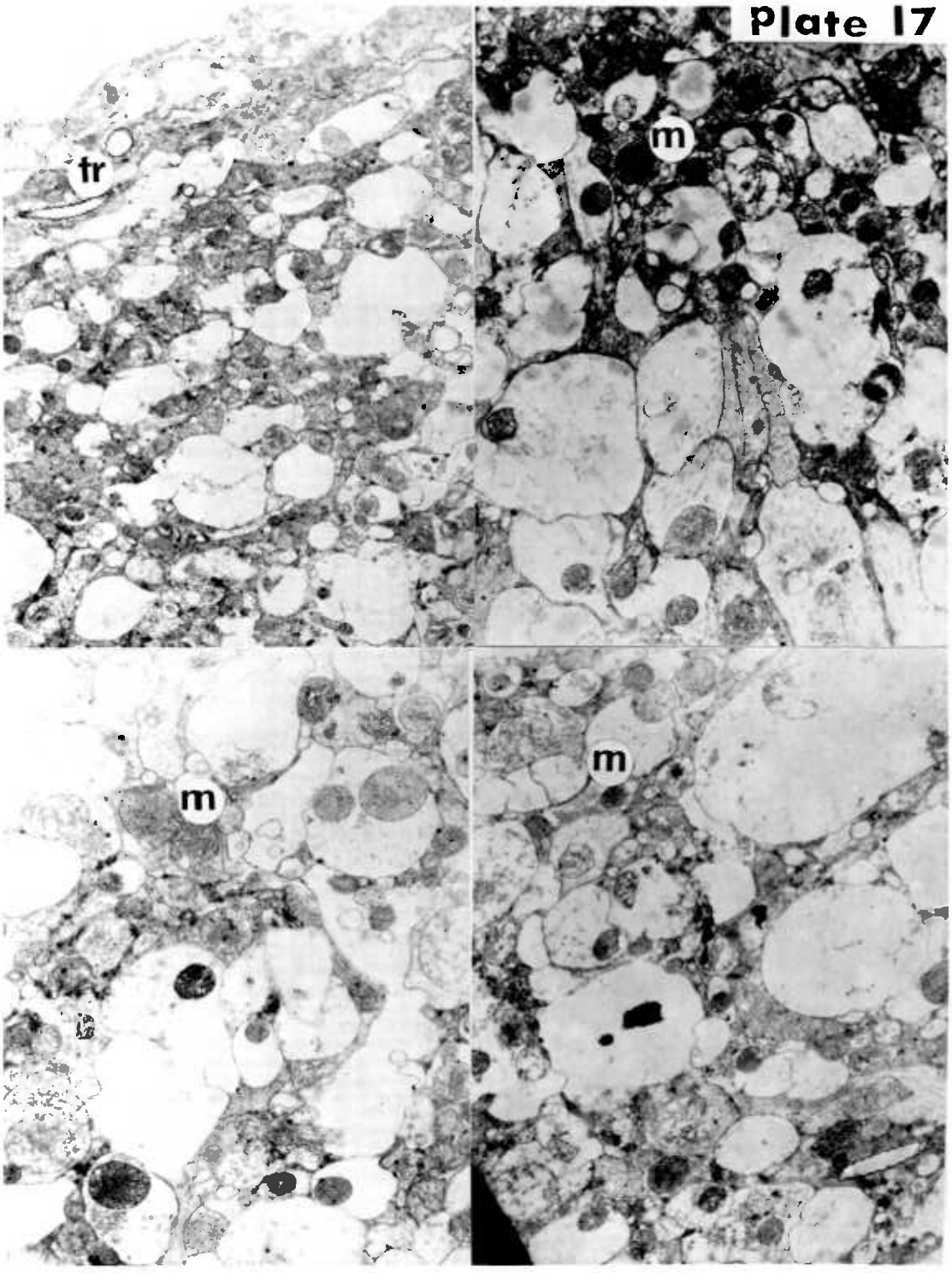


Plate 17
tr
m
m
m

PLATE 18

ELECTRON MICROGRAPH SHOWING THE MICROTUBULES AND MITOCHONDRIA FROM THE CAPSULE OF ADULT BEETLES.

Key

	microtubules arrowed
m	mitochondria
mt	microtubules

(X 79800)

Plate 18

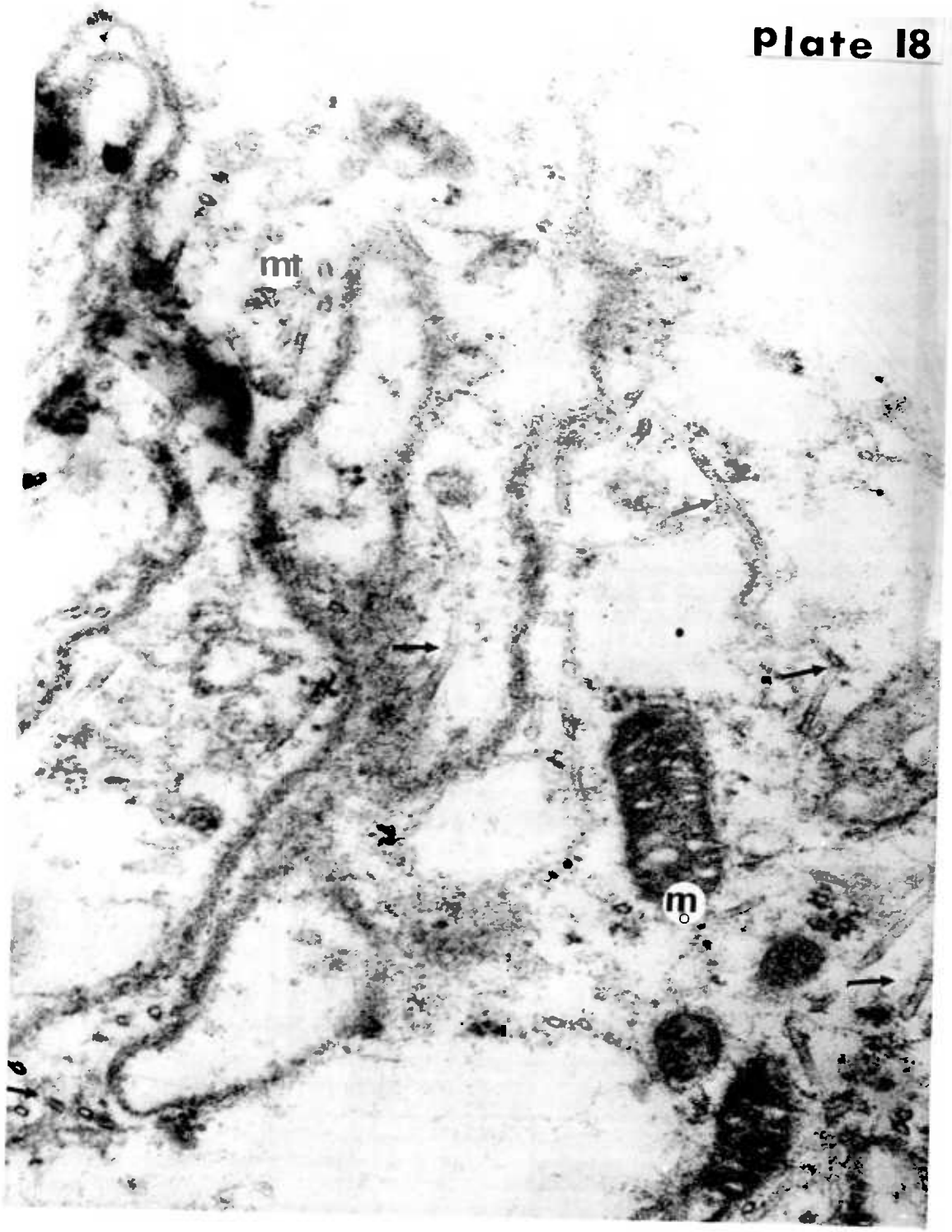


PLATE 19

ELECTRON MICROGRAPH SHOWING THE MICROTUBULES AND MITOCHONDRIA FROM THE CAPSULE OF ADULT BEETLES.

Key

microtubules arrowed

m mitochondria

mt microtubules

(X 79800)

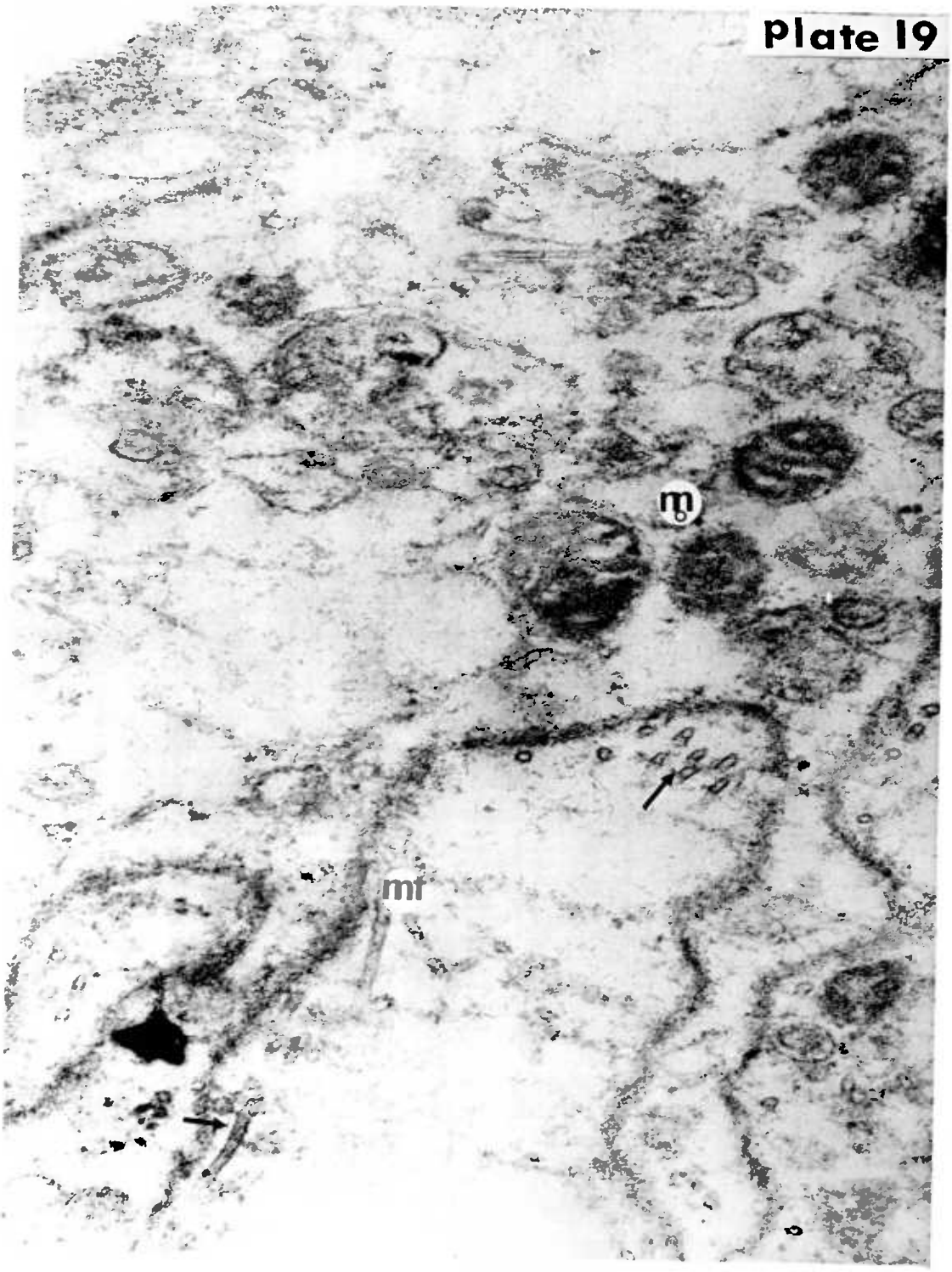


Plate 19
m
mf

12, 13 and 23).

Many variations were found in the structure of the capsules from young adults. In the inner region, the capsule appeared homogenous and were highly packed with numerous mitochondria of normal and abnormal structure. Some had swollen while others had irregular cristae. Nuclei of abnormal shape were found in this region. Membranous limited, electron dense bodies were abundant in this region, presumably they were the material extruded from the nuclei surrounded by the membranes from the collapsed nuclei (Plates 7, 16 and 17). Lysosome like bodies, microtubules, golgi bodies, trachea and fine tracheoles were also found (Plates 16, 17, 18 and 19). While capsules from adult beetles contain the female nematode and her progeny, in old beetles the nematode larvae had escaped as third stage larvae from the capsules. These capsules appeared non-cellular and non nucleate, the only structures remaining were the trachea and tracheoles and distinct regions or zones can no longer be distinguished (Plates 13 and 23). Tracheae and tracheoles were present in abundance, ramifying through the whole area, whorls of membranes were abundant presumably they are the membranes of degenerated nuclei, but few mitochondria and other structures were seen. Dense bodies of

PLATE 20

SECTIONS THROUGH THE CELLULAR CAPSULE FROM OLD ADULT BEETLES, SHOWING THE DEGENERATING AND COLLAPSING NUCLEI

Key

cn	collapsed nucleus
db	dense bodies
dn	degenerating nucleus (shown as up)
er	endoplasmic reticulum
m	mitochondria
pm	membrane pocketings
	microtubules arrowed

(X 6384) top

(X 4256) middle

(X 17955) bottom

Plate 2o

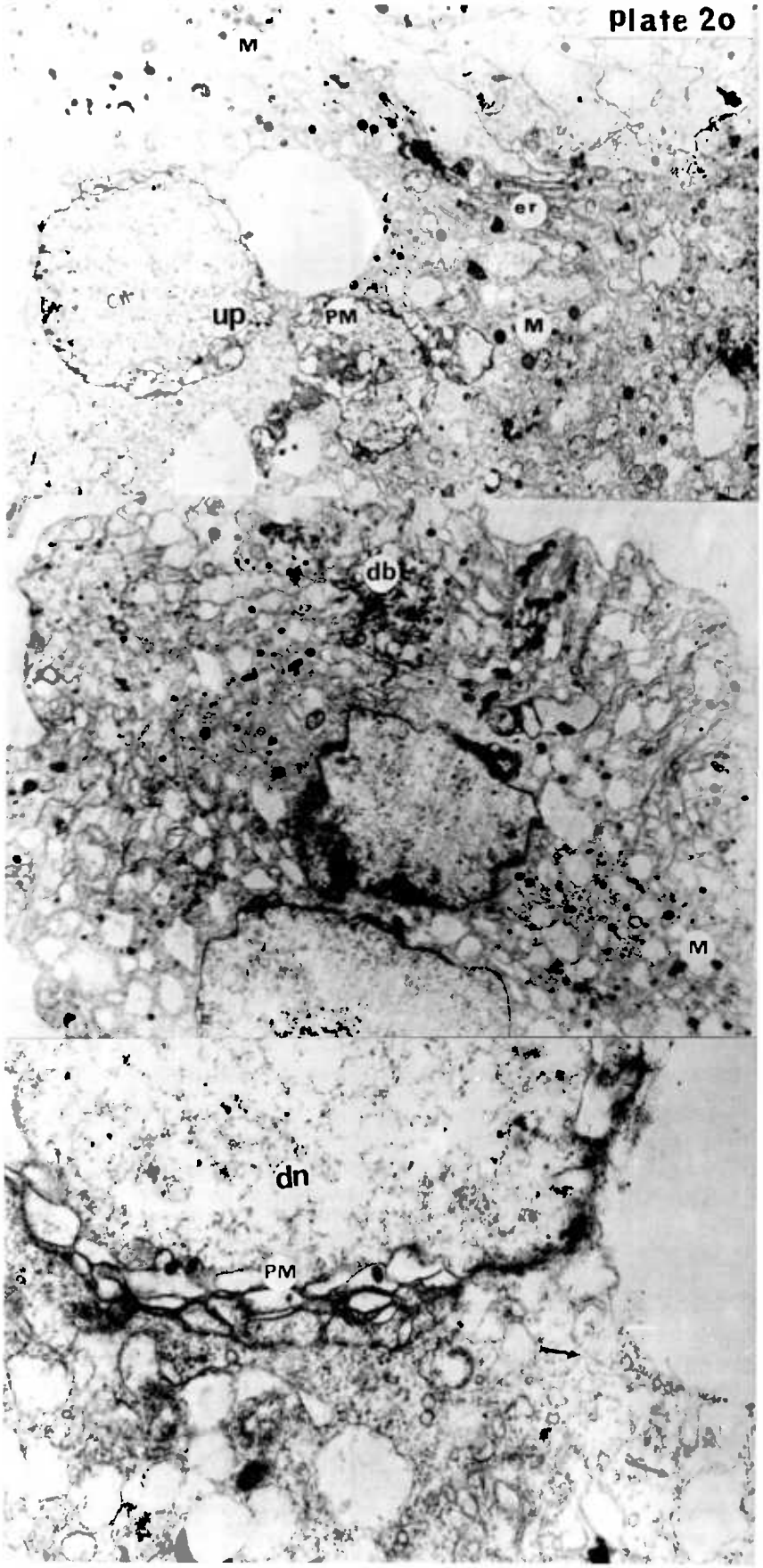


PLATE 21

SECTIONS THROUGH THE CELLULAR CAPSULE FROM OLD ADULT BEETLES, SHOWING THE DEGENERATING AND COLLAPSING NUCLEI

Key

cn	collapsed nucleus
db	dense bodies
dn	degenerating nucleus (shown as up)
er	endoplasmic reticulum
in	indistinct nuclear membrane
	microtubules arrowed

(X 20520)

Plate 21

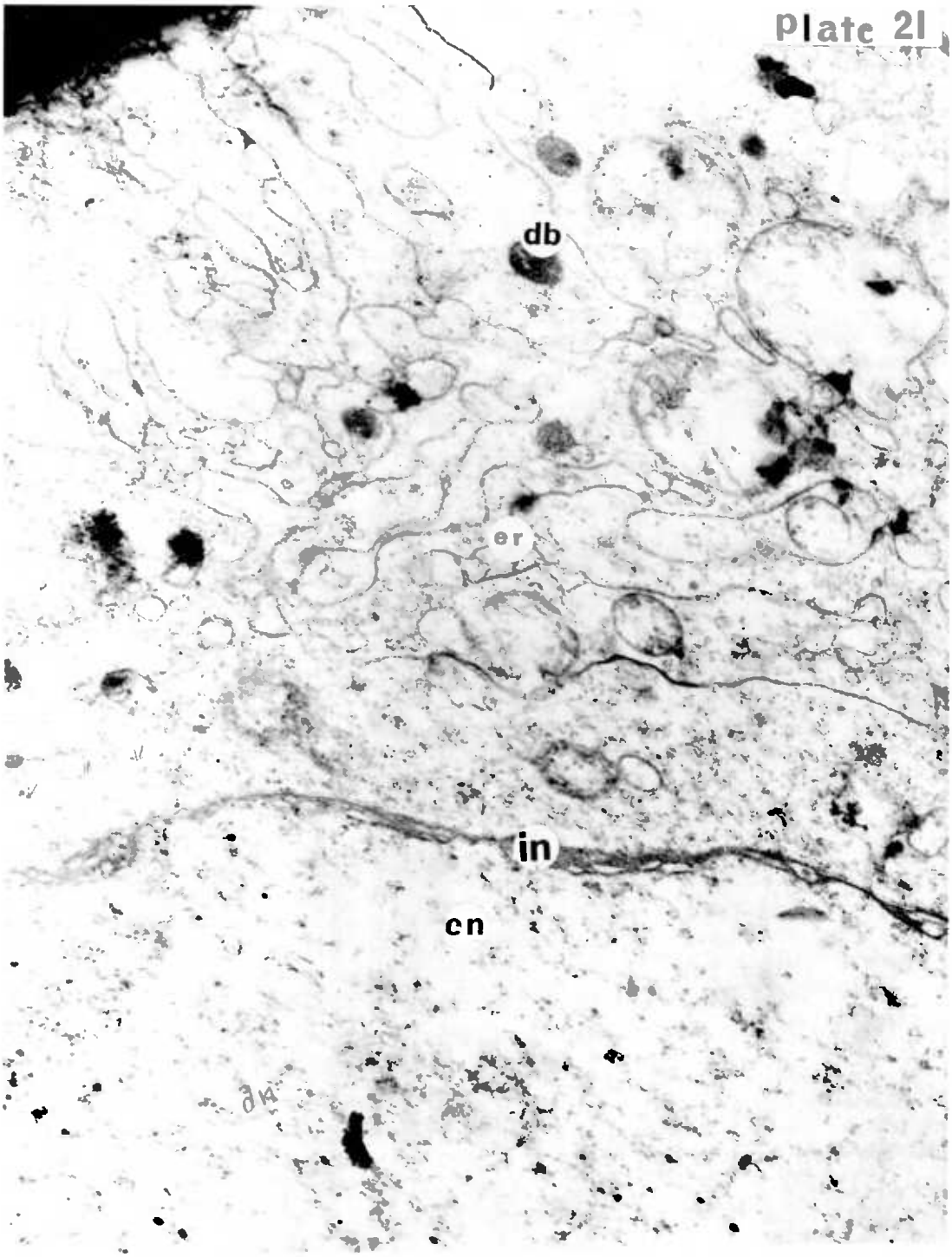


PLATE 22

SECTIONS THROUGH DIFFERENT CELLULAR CAPSULES DISSECTED FROM PARASITIZED ADULT BEETLES SHOWING TRACHEATION.

top left and right
CAPSULES FROM YOUNG ADULT HOST BEETLE AFTER THE INITIATION OF TRACHEATION
(X 1295014) top left
(X 20520) top right

bottom
A CAPSULE FROM OLD ADULT HOST, SHOWING THE TRACHEA AND TRACHEOLES OUTSIDE AND WITHIN THE CAPSULE WALL
(X 13680)

Key

nu nucleus
no nucleoles
tr tracheoles scattered all over the capsule tissue
TR Trachea

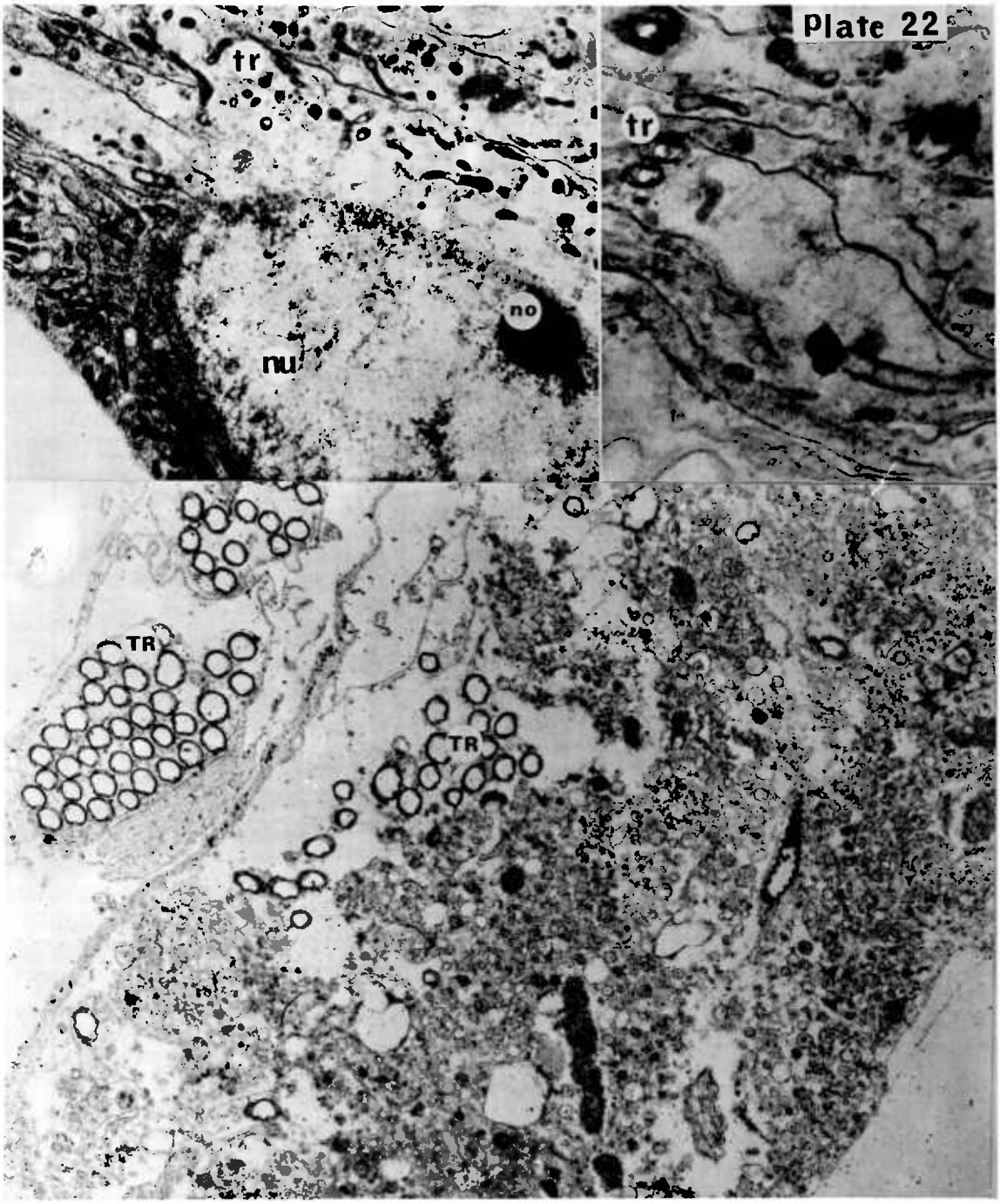


Plate 22
tr
tr
no
nu
TR
TR

PLATE 23

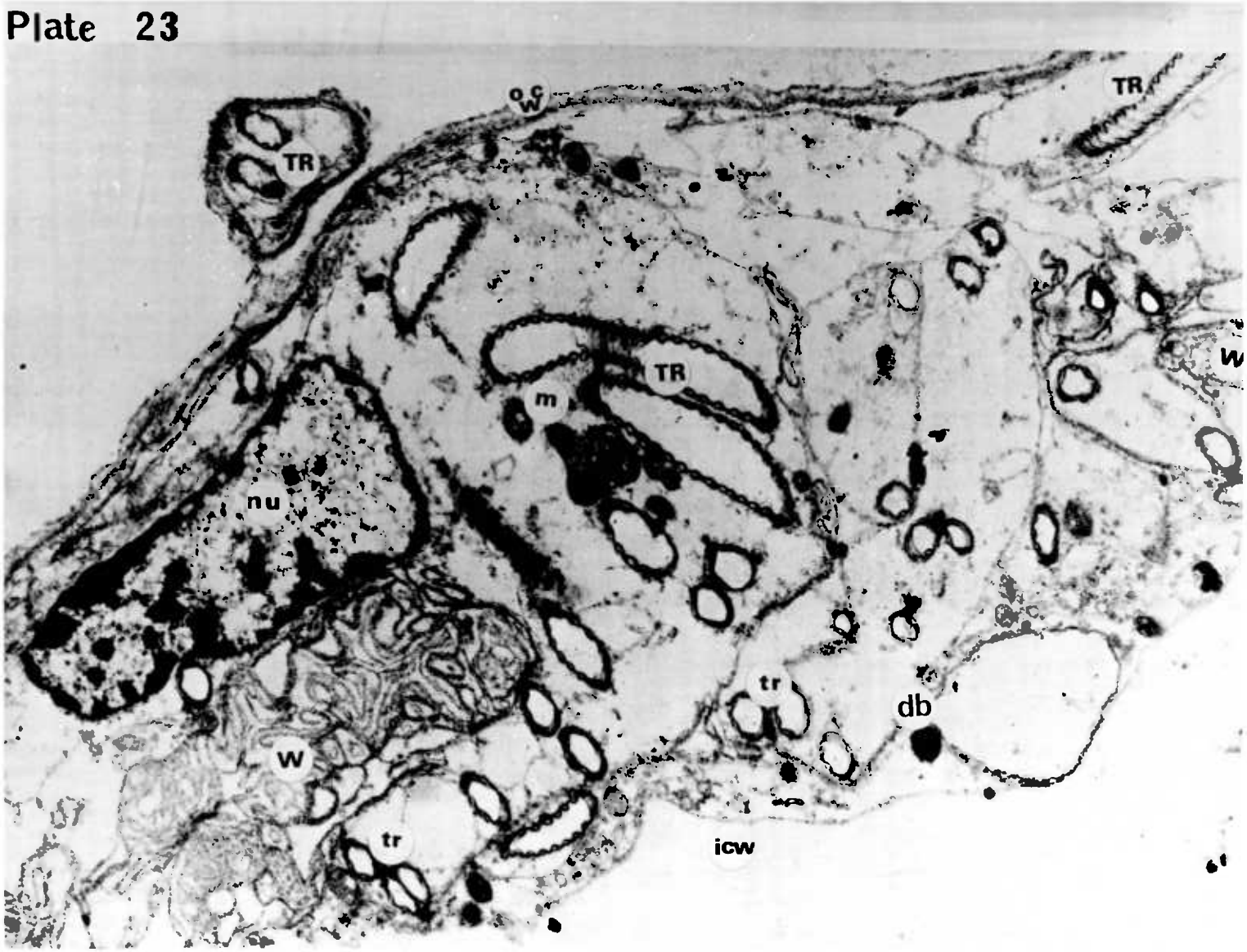
A CAPSULE FROM AN OLD ADULT BEETLE SHOWING ITS TRACHEATE STRUCTURE

Key

db	dense body
icw	inner capsule wall
ocw	outer capsule wall
nu	nucleus
m	mitochondria
TR	Trachea
tr	tracheoles
W	Whorls of membranes

(X 43890)

Plate 23



different sizes, possibly lysosomes, were in abundance (Plates 13 and 23).

The capsules were at first irregular in shape (Plate 24) particularly in the pupal host and they were thin, transparent, or off-white in colour, they contain the young immature parasitic females. The number of adult nematodes in the capsule apparently determined its size (Plate 25).

Capsules in the imagos were spherical in shape, with a smoother outline, often thicker and lighter brown in colour. They contained the mature parasitic females, her eggs and larvae. The capsules here were bigger than those from pupal hosts, even when they contained the same number of adult parasites (Plates 26 and 27). The capsule often appeared lamellate in cross section due to the flattening of the cells (Plate 28).

Trachea were frequently observed to become attached and even cover the capsules externally. Small tracheoles ramified and embedded in their tissues. Up to 90% of the capsules were found to contain tracheae and tracheoles.

The capsules which vary in size, were proportional to the parasite number per capsule, and to the parasites burden of the host, indicating the number of capsules per host (Table 4).

PLATE 24

CAPSULE FROM PUPA, CONTAINING ONE ADULT NEMATODE, (IRREGULAR) (X 100)

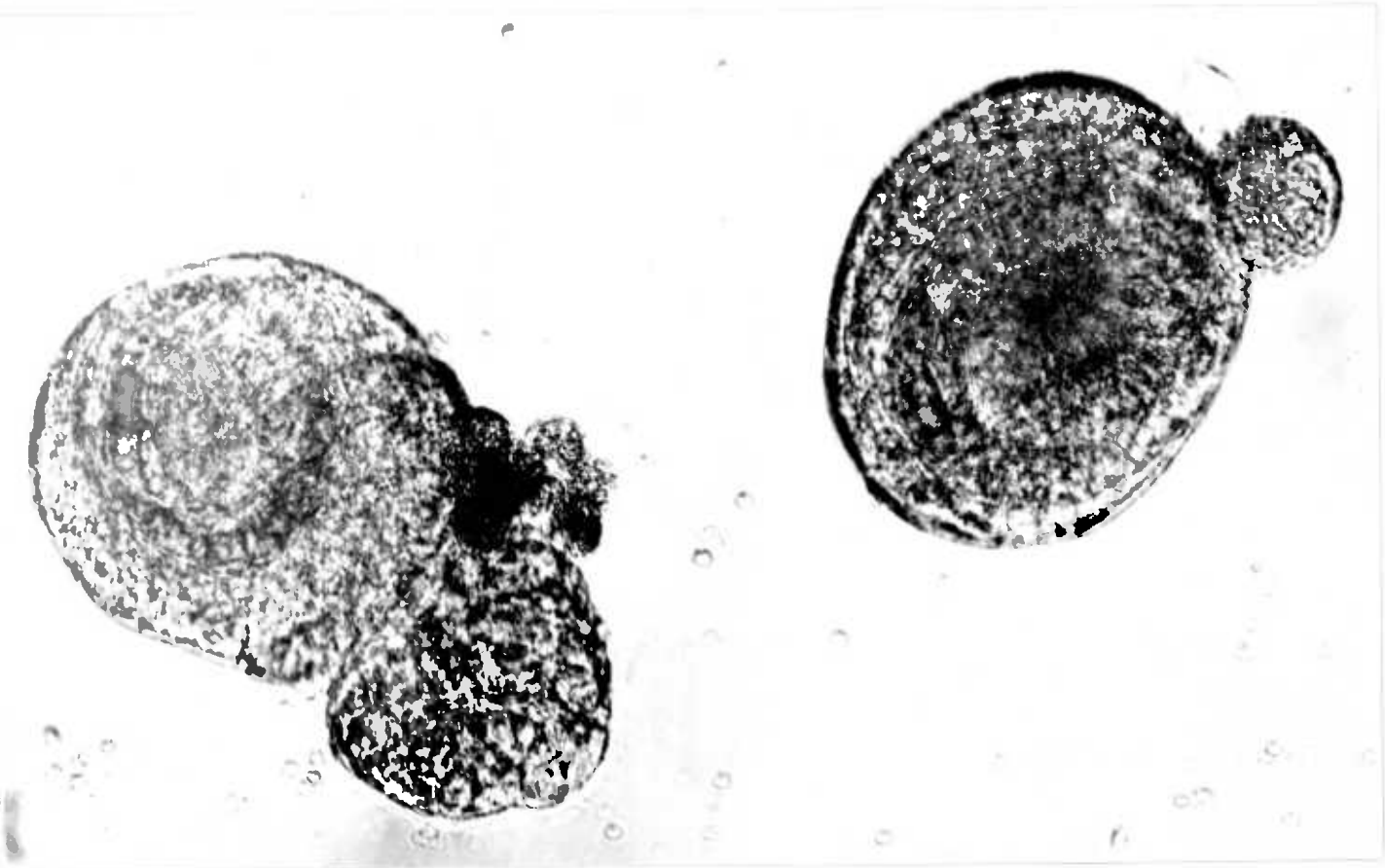


PLATE 25

CAPSULE FROM PUPA, CONTAINING MORE THAN ONE ADULT FEMALE NEMATODE (X 100)

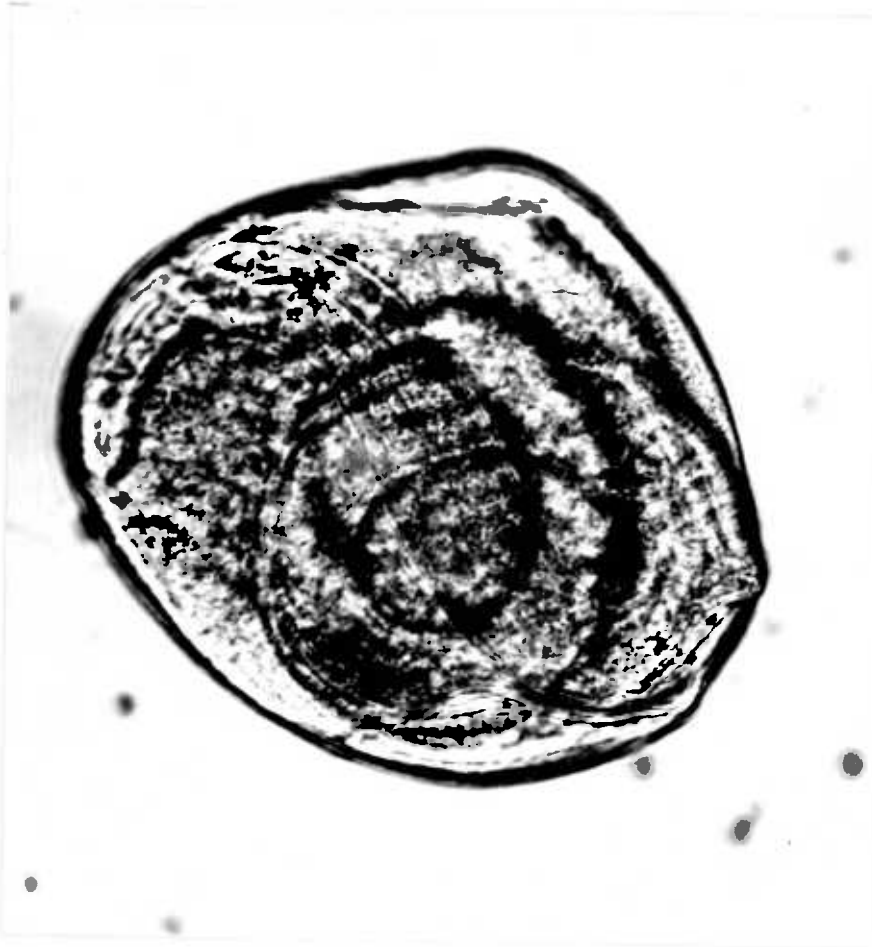


PLATE 26

CAPSULE FROM ADULT BEETLE CONTAINING ONE ADULT FEMALE NEMATODE WITH ITS' PROGENY.

(X 100)

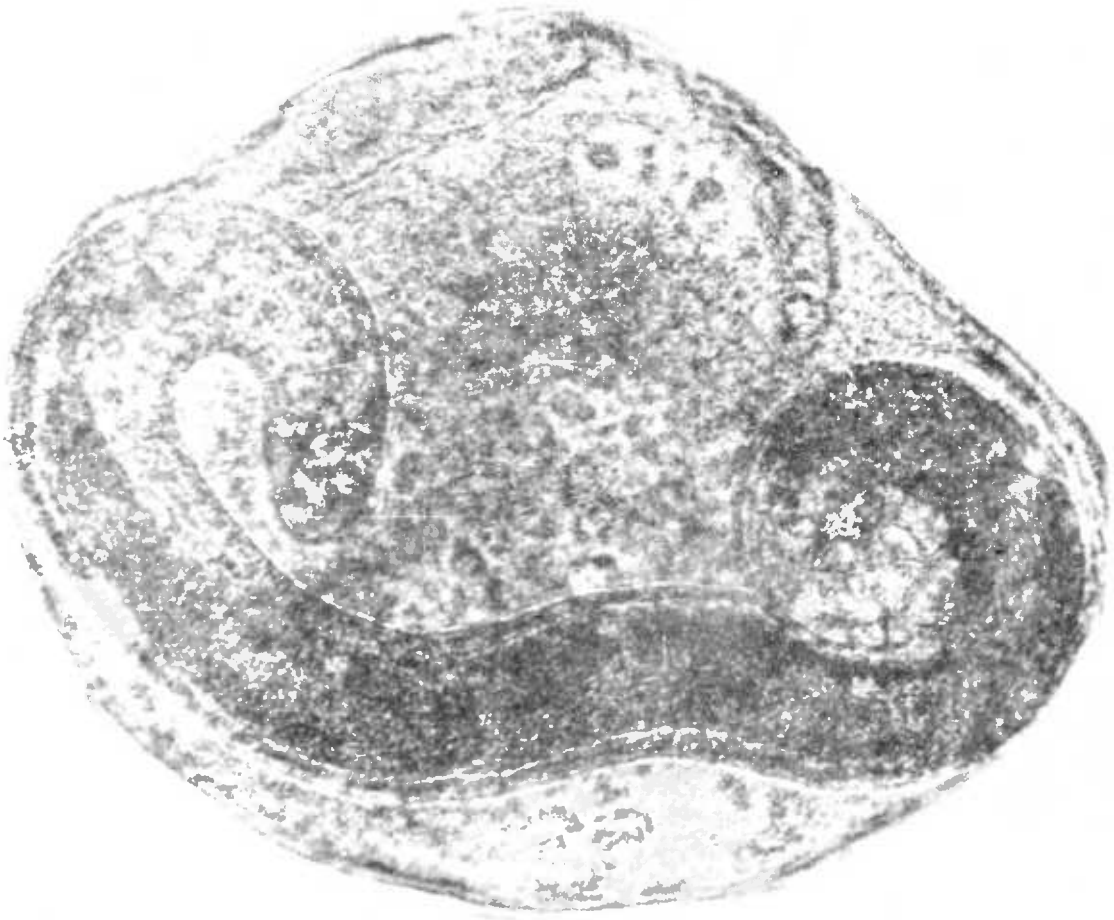


PLATE 27

CAPSULE FROM ADULT BEETLE CONTAINING FIVE ADULT FEMALE NEMATODES WITH THEIR PROGENY (X 100)

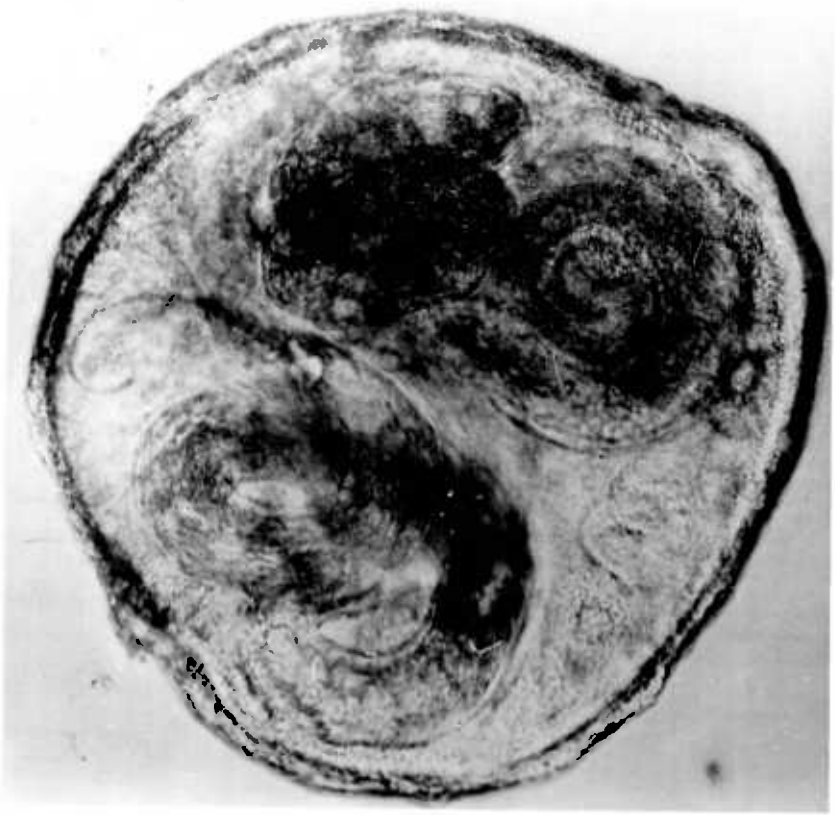
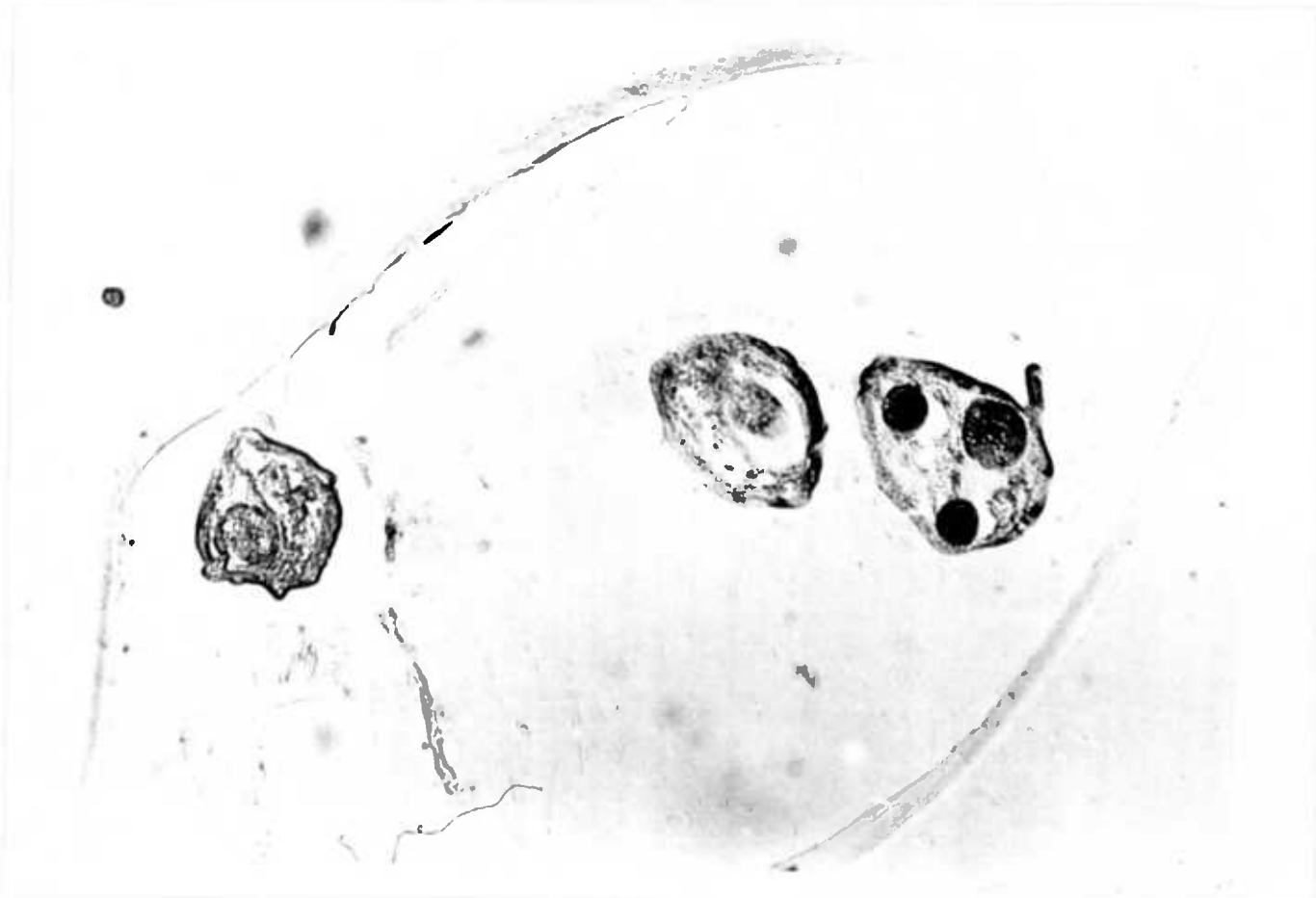


PLATE 28

CROSS SECTION OF THE CAPSULE SHOWING ITS LAMELLATE SHAPE AND SECTIONS OF THE NEMATODE (X 100)



The volumes of the capsules varied considerably. Capsules with up to 8 parasites (Plate 27) were bigger than ones containing only a single parasite. These capsules had thinner walls in the hosts with more than one parasite.

In the adult beetle, as the number of parasite per host increased, the extent of encapsulation decreased. This was an inverse relationship to the degree of super-parasitism. Capsules without tracheae or tracheoles were only found in hosts with a very low density of parasites, usually fewer than 3, and in the pupal host the tracheoles are less well developed.

Complete encapsulation was always observed in the pupal host (Fig. 6) regardless of the number of parasites per individual host. The percentage encapsulation declined as the parasites matured. After egg laying, all the progeny having developed to third stage larvae, left the capsules.

Table 4 The effect of different parasite densities on the capsule volume in pupae and adult beetles

Host	No. of parasites/capsule	No. of capsules/host	(μm) major axes of the capsule (Mean)	(μm) minor axes of the capsule (Mean)	(μm^3) mean volume of the capsule $\times 10^5$
Pupae	1	1	249	188	61
	3	1	364	303	210
	5	1	523	328	469
	1	1	360	264	179
	1	2	270	234	89
	1	3	264	230.5	84
	1	4	238.5	154.5	45
	1	7	203.8	123.7	26
Adults	1	1	348	300	190
	3	1	420	360	332
	5	1	528	432	630

The incidence of encapsulation in adult hosts ranged between 19 - 75 percent (Fig. 6). There was a linear relationship between parasite density and the percentage encapsulation and the 'best fitting' regression line was calculated (appendix for calculation). Using Student's 't' test for significance, it was found that the

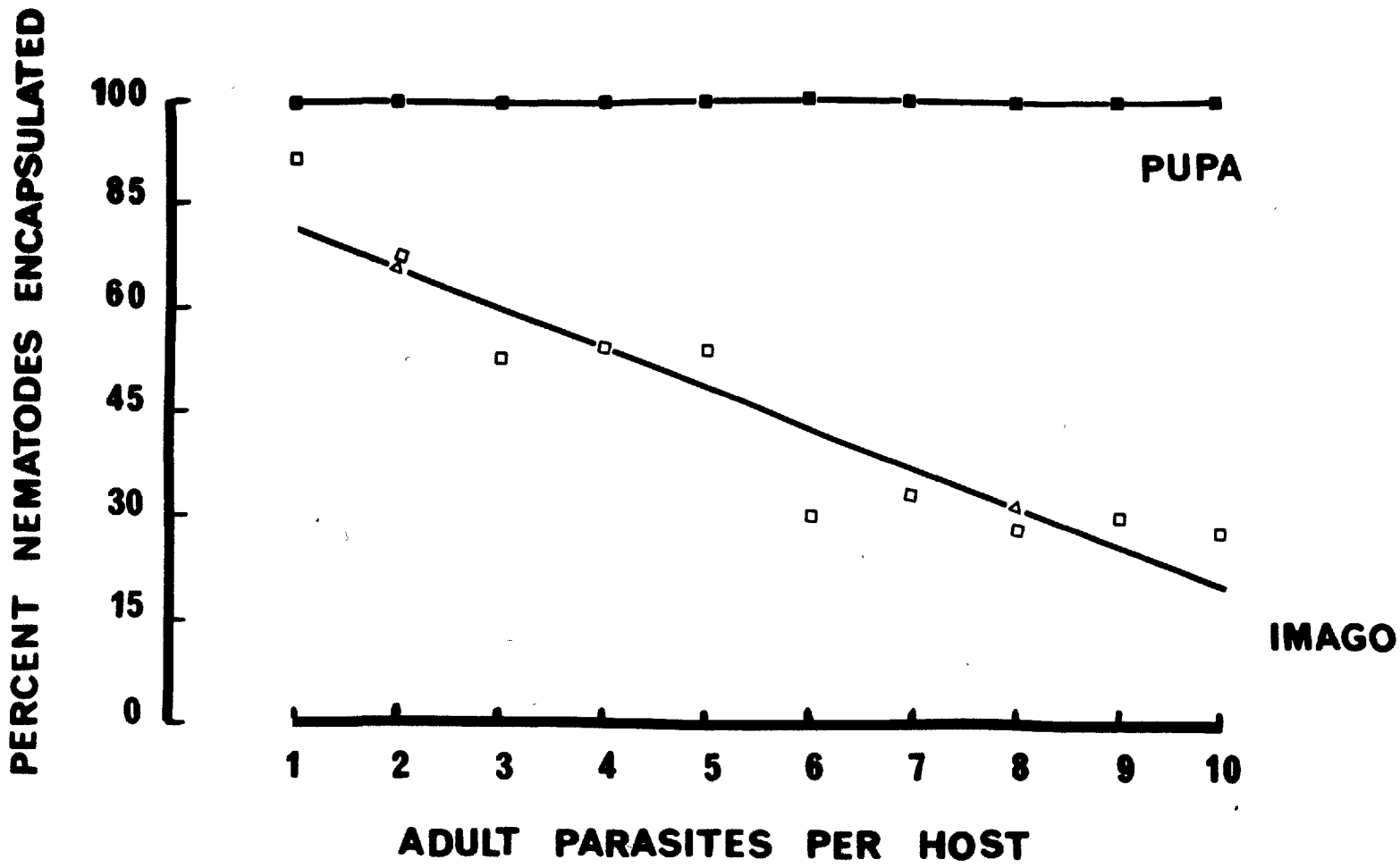
probability of observing a value of $\underline{t}$ with 8 degrees of freedom greater in absolute value than 5.041 is 0.001. Table App. showed that $t_8 = 6.808$ which is greater than 5.041, so the probability of obtaining this result by chance is less than one in a thousand.

FIG. 6

THE EFFECT OF PARASITE DENSITY ON THE EXTENT OF THE ENCAPSULATION

Key

Δ equation point


PERCENT NEMATODES ENCAPSULATED
100
85
60
45
30
15
0
PUPA
IMAGO
1
2
3
4
5
6
7
8
9
10
ADULT PARASITES PER HOST

3.5. The effect of single and multiple infection on the size of the developing parasites

The volume of the parasite was found to decrease with an increase in number of adult female parasites present in the haemocoel of the beetle (Appendix table 2) (Fig. 7). There was a linear relationship between the number of parasites per host and the percentage reduction in volume of these parasites when compared to the calculated volume of parasites from single infections. When there was only one adult female in the host, it was considerably bigger than those from highly infected hosts; the smallest changes occurring when there were only two parasites per host. Individual parasites occurring alone are bigger than individuals from fequency groups such as four or five parasites per host. Fig. 7 and table 2 app. also showed that the reduction in volume of a parasite occurring in groups of 8 parasites per host was greater than specimens occurring in groups of four. A test for significance was made using Student's 't' test, it was found that the probability of observing a value of t with six degrees of freedom greater in absolute value than 5.2, $P = 0.002$. The t_6 value was 5.799 which is significant, since it is greater than 5.208; the probability of obtaining this result by chance is less than two in a thousand.

FIG. 7

DECREASE IN PARASITE VOLUME IN MULTIPLE INFECTIONS.

Key

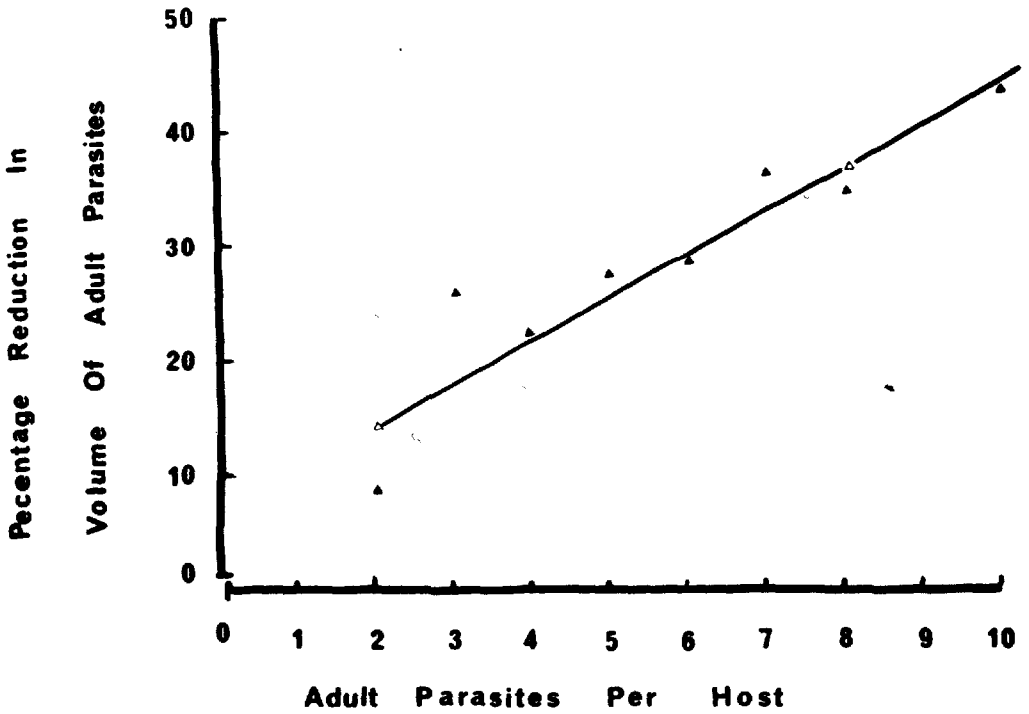
△ .. △ . . . equation points

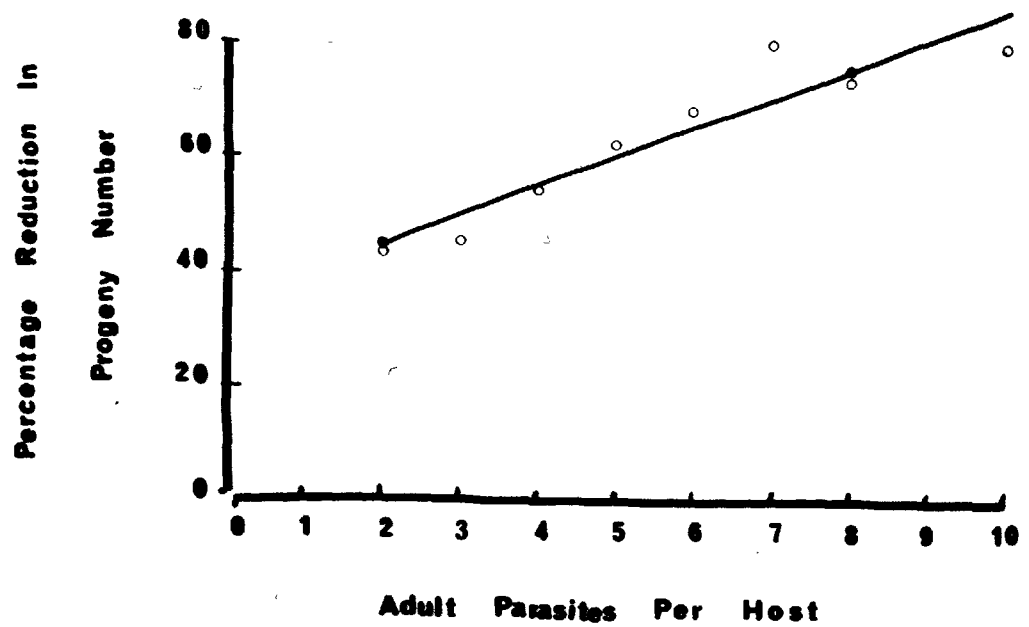
FIG. 8

DECREASE IN PARASITE FECUNDITY IN MULTIPLE INFECTIONS.

Key

● ● equation points


Pecentage Reduction In
Volume Of Adult Parasites
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Adult Parasites Per Host


Percentage Reduction In
Progeny Number
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
Adult Parasites Per Host

3.6. Effect of single and multiple infection on parasite fecundity

The results (Appendix table 3) showed that there was a reduction in the mean offspring produced by each female parasite as the number of parasites per host increased. Thus the percentage reduction increased with increasing numbers of parasites per beetle. The difference in number of progeny produced by parasites occurring in groups between 7 and 8 is very low compared with the number of progeny occuring alone as in group of two. Fig. 8 showed a linear regression between the number of parasite per host and the percentage reduction in progeny. A test for significance was done using Students 't' test, it was found that value of t for 6 degrees of freedom was greater in absolute value than 5.959, so the probability of observing value of t is 0.001. The value of t_6 was 6.916 which is highly significant value since it is greater than 5.959, and the probability of obtaining this result by chance is less than one in a thousand (Appendix table 4).

3.7. The relationship between the development of the capsule and the development of the parasite

The host reaction to nematode infection always resulted in the formation of the capsule; the formation

of the capsule was associated with the host's haemocytes seems to show successful host-parasite relationship by Contortylenchus and Ips sexdentatus. The nematodes obtained sufficient nourishment to mature and produce hundreds of eggs and larvae. The host haemocytes that form the capsule undergo different modifications so all the cell membranes breakdown or lyse and the cytoplasmic contents mix to form the envelope around the nematode which is full of big nuclei, as a multinucleate syncytia.

The nematodes grow and reproduce inside the capsule; the growth of the parasite being very fast in the capsule from pupal host (Fig. 9) until the parasite becomes mature and begins to lay eggs, in the young adult host. The capsule volume increased steadily as a result of this growth, and continued increasing as the nematodes developed and reproduced. The maximum capsule volume was $217 \times 10^5 mm^3$ from an adult beetle. This occurred when the capsule contained eggs and larvae (Fig. 9 I C) in different stages of development. It may be possible that this increase in size of the capsule was caused by the activity of the contained larvae which stretched the capsule wall or the osmotic relationship between nematodes and capsule. When the larvae had left the capsule, the capsule size declined (Fig. 9 I D). The relationship

between the ratio of the total volume of nuclei to the ratio of mean volume of the capsules in each group and the development of the parasite was shown in fig. 9 III. The ratio was greatest at the pupal stage when the nuclei were bigger than at any other time. The enlarged nuclei then tended to be reduced in size at the time when the parasites completed their development and reproduced. In the 2nd group of capsules when they contain larvae and eggs the ratio was 100 times less than that at the first group of capsule and the ratio continued to decline in the last group (Fig. III D). The relationship between the development of the parasite (Fig. 9 II) and the number of nuclei, indicated that the highest number of nuclei were obtained from young capsules and as the parasites developed the number of nuclei diminished. Although more haemocytes were attached to the capsule, during the adult hosts development a reduction in number of nuclei occurred. Presumably this was caused by the utilization of nuclei during the development of the parasite. The number of nuclei was reduced to 2 nuclei in very old capsules, or became completely non-nucleate.

Fused nuclei are often seen at all stages of the capsule's development (Plate 29 B). Plate 29 showed all

FIG. 9

THE RELATIONSHIP BETWEEN THE DEVELOPMENT OF THE CAPSULE AND THE DEVELOPMENT OF THE INCLUDING NEMATODE

I. Volume of the capsule during its growth in different developmental host stages

II. Number of Nuclei during the capsule growth in different developmental Host stages.

III. The ratio of the volume of capsule's nuclei to the volume of the capsule during its growth in different developmental host stages.

Ordinates -

I. Mean volume of the Capsule (mm^3) each number multiplied by (10^5)

II. Mean number of nuclei per capsule.

III. The ratio between the mean volume of the nuclei to the mean volume of the capsule.

Abscissa

The developmental stages of the nematodes within the capsule

A. Young adult parasitic female nematode in a capsule from host pupa.

B. Mature adult parasitic female nematode in a capsule (with eggs only) from young adult host beetle.

C. Adult parasitic female in a capsule (with eggs and larvae) from adult host beetle.

D. Adult parasitic female in a capsule (no eggs, no larvae) from an old adult host beetle.

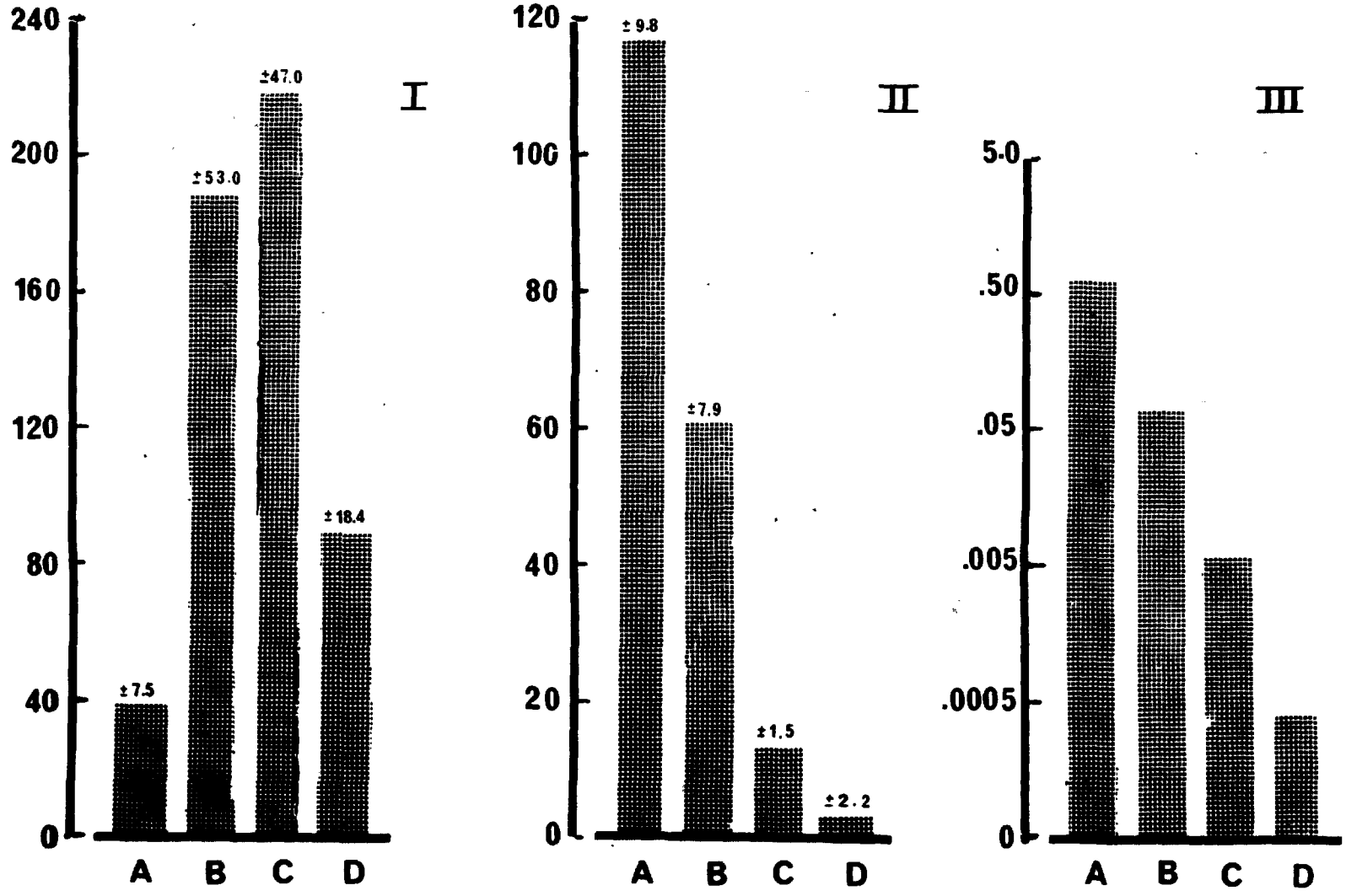

I
240
200
160
120
80
40
0
±7.5
±53.0
±47.0
±18.4
A
B
C
D
II
120
100
80
60
40
20
0
±9.8
±7.9
±1.5
±2.2
A
B
C
D
III
5.0
.50
.05
.005
.0005
0
A
B
C
D

PLATE 29

CAPSULES IN DIFFERENT STAGES OF DEVELOPMENT STAINED WITH FEULGEN STAIN FOR DNA

A. Capsules from pupal host showing the stained nuclei only.

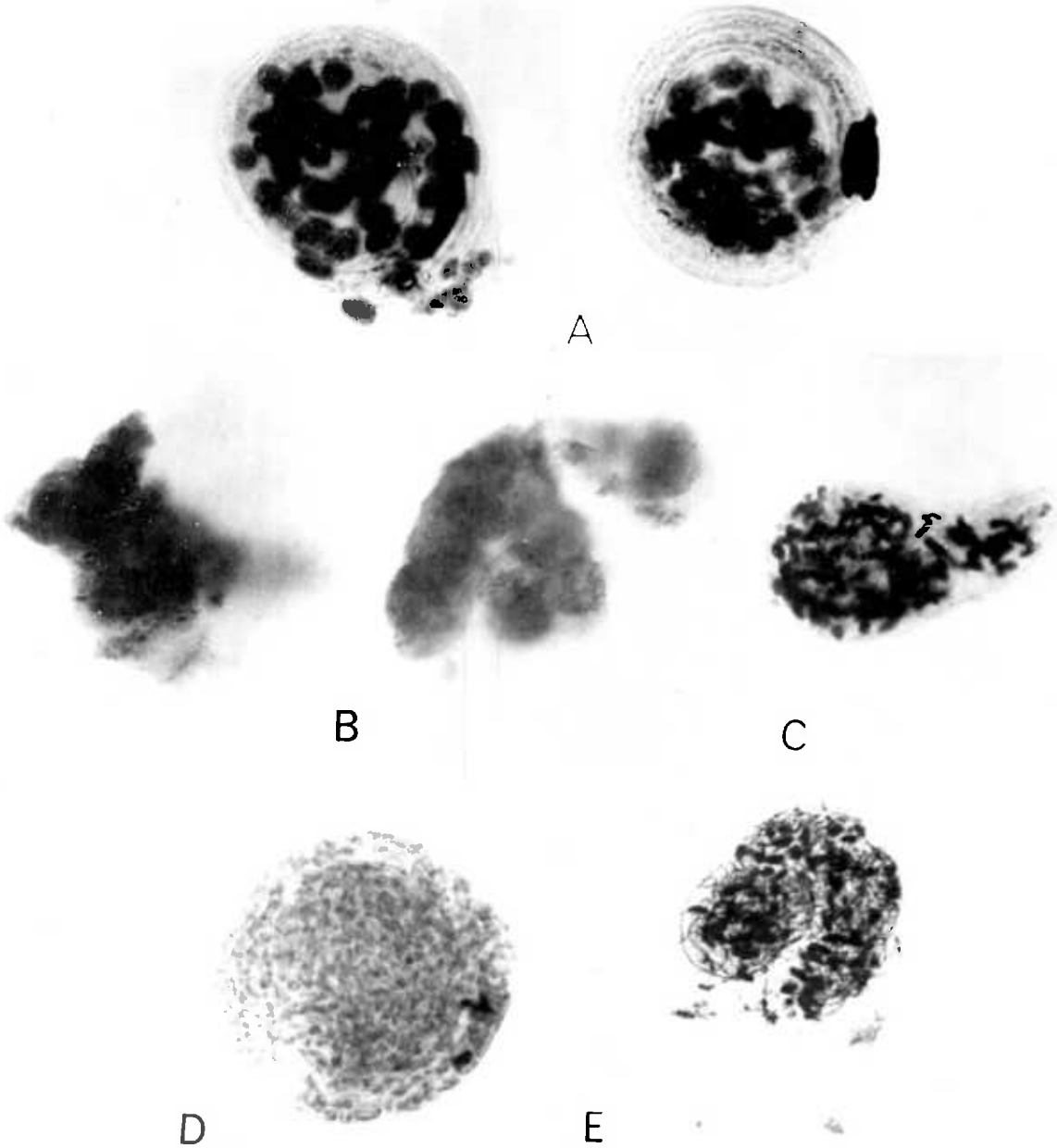
B. Nuclear fusion during the growth of the capsules.

C. Capsules from young adult host contained nematode's eggs only.

D. Capsule from adult host contained eggs and larvae of the nematode.

E. Capsule from an old adult host beetle showing the larvae leaving the capsule.

(X 100)


A
B
C
D
E

the different stages of the capsule's development. The capsule from the pupal host (Plate 29 A) contained only the developing female nematode surrounded with nuclei, while capsules from young adult beetles contained the mature adult female and her eggs and few nuclei can be seen (Plate 29 C). Nuclei can just be seen in the capsules from adult beetles when they contained the adult female nematode and her progeny. Eggs and larvae were in capsules when the larvae started leaving them as third stage larvae (Plate 29, D and E).

3.8. The Relationship between the intensity of parasitism and the morphometrics of the adult parasites

During the dissection of the beetle host, to get the parasites for experimental purposes, it was stated that measurements for 292 living specimens were made; consequently the data was arranged into groups related to the number of parasites per host.

The increased number of parasites per host was found to result in a decrease in length and width of the mature parasite. The dimensions, ie. length and width of parasites in the range of 1-10 parasites per host, were studied. The number of replications for each parasite density varied between 4 - 10. The summary of the data is given in appendix (Table 2).

The mean length and mean width of the parasite from each parasite density was determined and the a value calculated. The data was illustrated as follows:

1. The mean length of the parasite from each parasite density/host was plotted against the mean nematode width (Fig. 10).
2. The mean length of the parasite was plotted against the parasite density/host (Fig. 11)
3. The mean a value of the adult parasitic female nematode calculated from nematodes obtained at each density were plotted against the parasite density per host (Fig. 12).

When there was but a single parasitic adult female per host, it was considerably larger than when several parasites were present in the same host. Value of a (ratio between length and width) was smaller than those for between 3 - 10 parasites/host, which indicates that the width of the parasite was effected by the density. The width and length of the parasite decrease as the burden of parasites in the haemocoel increases, length decreased more than width, Table 5.

FIG. 10

THE RELATIONSHIP BETWEEN THE LENGTH OF ADULT FEMALE NEMATODES IN ADULT BEETLES AS THE PARASITE BURDEN WAS INCREASED. NEMATODES FROM 1 UP TO 10/HOST WERE COMPARED.

Key

△ △ equation points

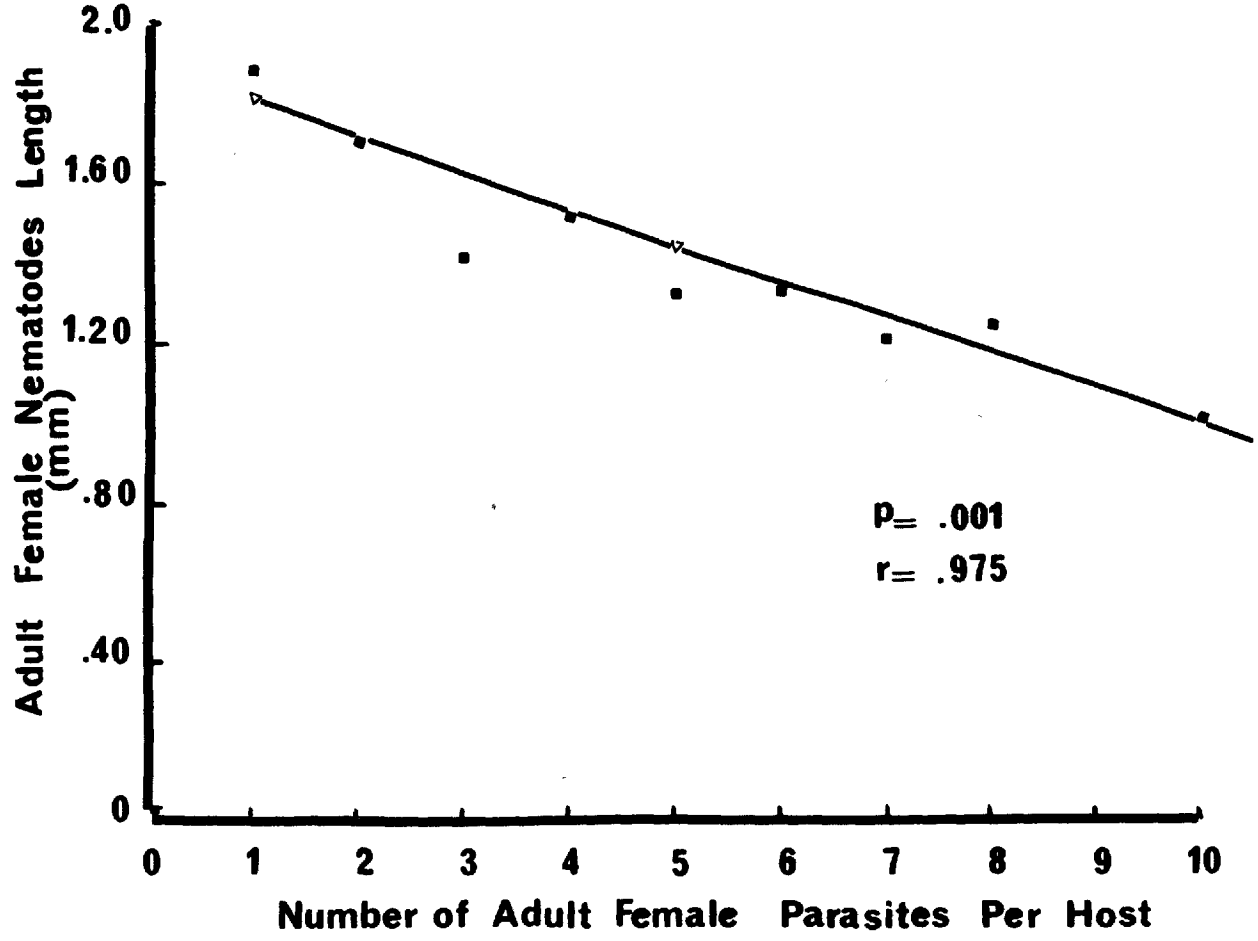

Adult Female Nematodes Length (mm)
2.0
1.60
1.20
.80
.40
0
0
1
2
3
4
5
6
7
8
9
10
Number of Adult Female Parasites Per Host
p= .001
r= .975

FIG. 11

THE RELATIONSHIP BETWEEN THE BODY LENGTH OF ADULT PARASITIC NEMATODES COMPARED TO THEIR BODY WIDTH. THE PARASITE MEASUREMENTS WERE OBTAINED FROM REPLICATED INDIVIDUALS AT DIFFERENT PARASITE DENSITIES/HOST.

Key

(1 - 10) indicate number of mature female parasites per host beetle.

O . O equation points.

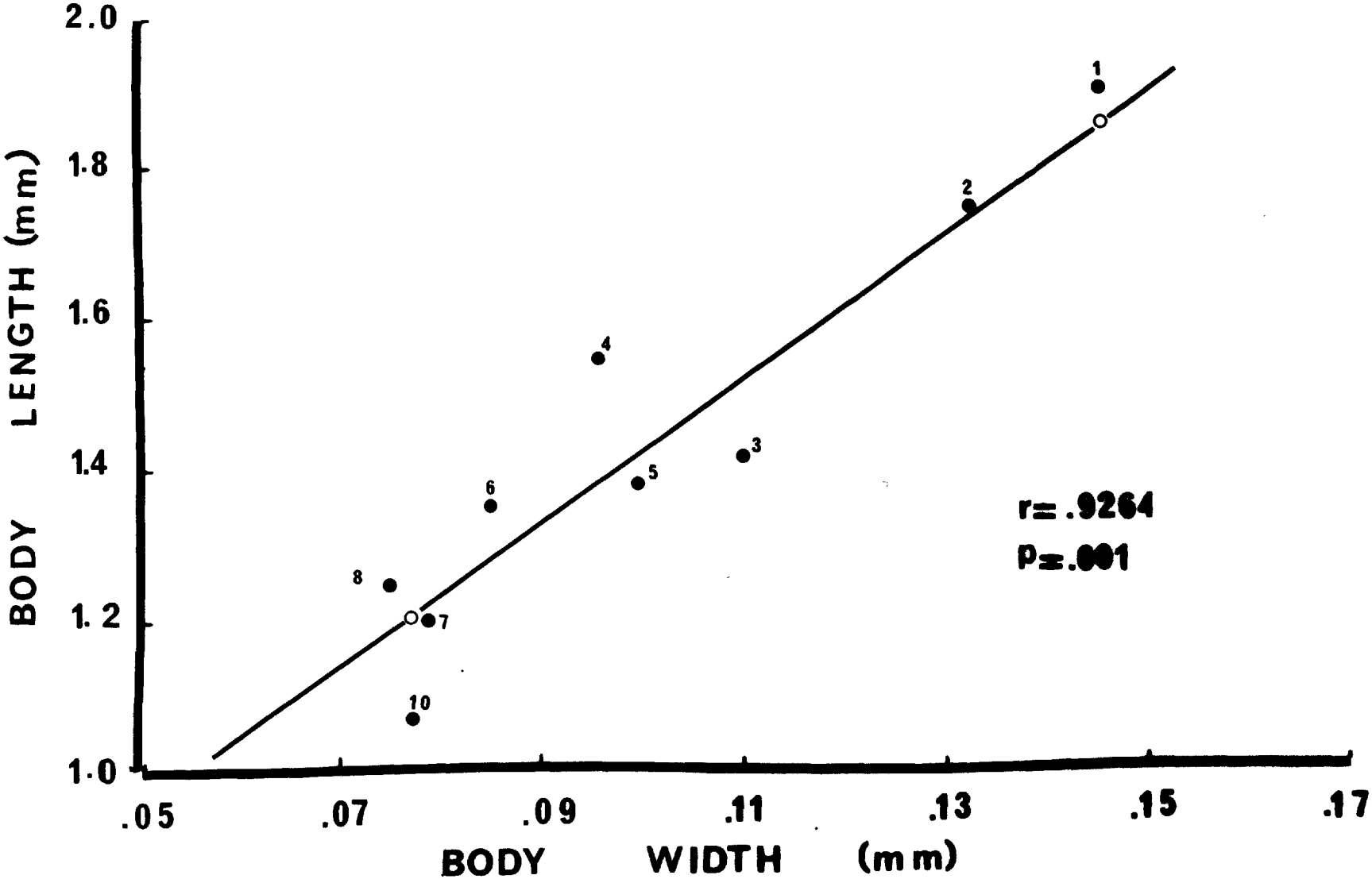

2.0
1.8
1.6
1.4
1.2
1.0
BODY LENGTH (mm)
.05
.07
.09
.11
.13
.15
.17
BODY WIDTH (mm)
1
2
3
4
5
6
7
8
10
r=.9264
p=.001

FIG. 12

THE EFFECT OF INCREASING PARASITE DENSITY ON THE ADULT BEETLE ON THE a VALUE OF THE ADULT FEMALE NEMATODES AT EACH PARASITE DENSITY.

Key

O O equation points.

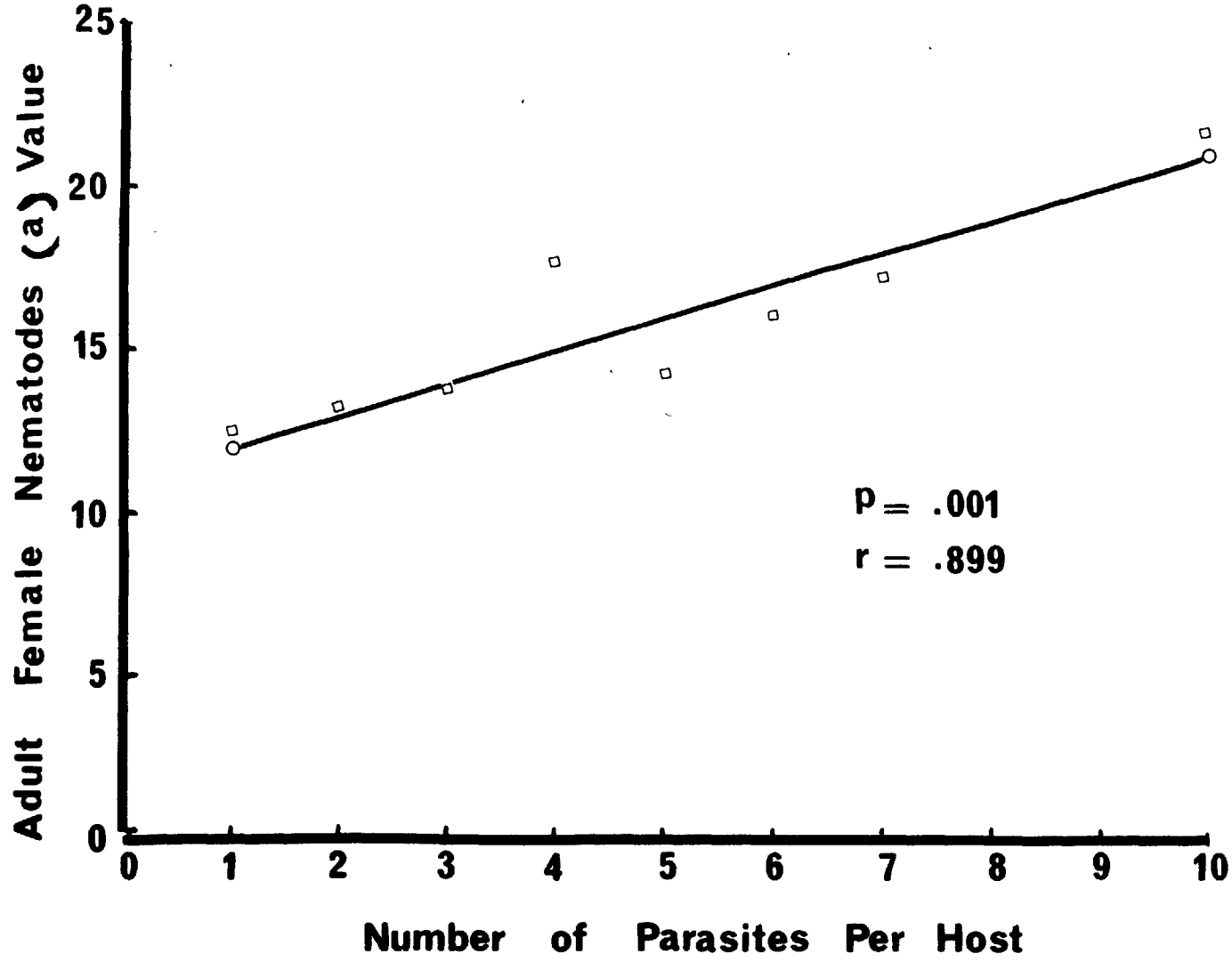

Adult Female Nematodes (a) Value
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
Number of Parasites Per Host
p = .001
r = .899

Table 5 Parasite Morphometrics in multiple infections

Number of parasites per host	Number of samples	Number of parasites measured	Mean Body length (mm)	Mean Body width (mm)	Value of a
1	10	10	1.89	0.146	12.526
2	10	20	1.73	0.133	13.38
3	9	27	1.41	0.115	13.96
4	9	36	1.54	0.096	17.85
5	7	35	1.37	0.100	14.03
6	7	42	1.35	0.085	15.99
7	6	42	1.20	0.079	17.23
8	5	40	1.25	0.075	19.16
9	-	-	-	-	-
10	4	40	1.07	0.077	21.88

There were linear relationships between the length and the width of the parasite according to its density per individual host (Fig. 10), between the length of the adult parasite and the number of the parasites per host (Fig. 11), and between the a value of the adult parasite and the parasite burden of each host (Fig. 12). However, the length and the width were greatly affected in multiple infections. After measuring 292 parasitic females the range in body length was found to be 0.996 - 2.04 mm, the width was ranging between 0.041 - 0.192 mm, and the a value ranged between 8.75 - 27.03. The mature

adult parasitic female nematodes are only found in the haemolymph of the host.

In this study a general impression was obtained that measurements for the parasitic female and all the progeny could throw a light on this species especially when these measurements compared with Ruhm's (1965) and Al-Rabiai's (1970).

From measurements of all the stages that live in the host, nematode morphometrics were obviously affected by the density of their own infection, not only the adult females but also their progeny. A table of the parasite measurements obtained in this study were compared to measurements from other published studies in an attempt to determine if this nematode was a new species, or could be synonymised with any other species of Contortylenchus. The measurements for all larval stages and the egg from the haemolymph, larvae from the gut and the free living stages from the Frass were as follows:

Eggs

egg length	(40 - 47 µm)	(inside the female)
" width	(12 - 16 µm)	
egg length	(54 - 62 µm)	(outside the female)
" width	(22.9 - 26 µm)	

Larvae in the eggs (1I)

L. = 158 - 166 µm (162 µm)

W. = 9 - 13 µm (10 µm)

L2

L. = 200 - 237 µm (218 µm)

W. = 14.5 - 16.6 µm (15.8 µm)

L3

L. = 296 - 345 µm (321.5 µm)

W. = 16.5 - 20 µm (18.9 µm)

From the gut (L4 male L = 496-586 µm (518 µm) W = 18-26 µm (21.0 µm)

(L4 female L = 482-566 µm (502.1 µm) W = 17-24 µm (22.0 µm)

Free living from the Frass:-

The male:

L 426 - 560 µm, W 15 - 17 µm

a = 33 - 38.5

The Female

L 526 - 592 µm, W 14.5 - 15.5 µm

a = 32.4 - 38.6

3.9. Incidence of parasitism and encapsulation in natural forest population and in laboratory reared stock

During the course of study and the dissections of beetles for many different experimental purposes, careful records were kept separately for each sample of wild beetle in order to get the data concerning the incidence of parasitism and encapsulation. The results obtained showed that the parasitism by nematodes of bark beetles varied considerably at different sampling dates and at the different sites. Hundreds of beetle larvae were found to harbour no nematodes, the greatest incidence of parasitism was less than 50%.

The incidence of infection from two different localities over different periods of time are shown in Fig. 13. The results showed that the incidence of parasitism in Yatley, reaching 44% in September, was higher than that of Alice Holt area, the highest incidence was 33.3% in April of the same year.

There was little difference between the sex of the beetle and the degree of infection, both are infested to the same extent, although males tend to contain a higher parasite burden than females.

The number of adult female nematodes parasitising the host varies between 1 and 60. A range of between 1 - 10

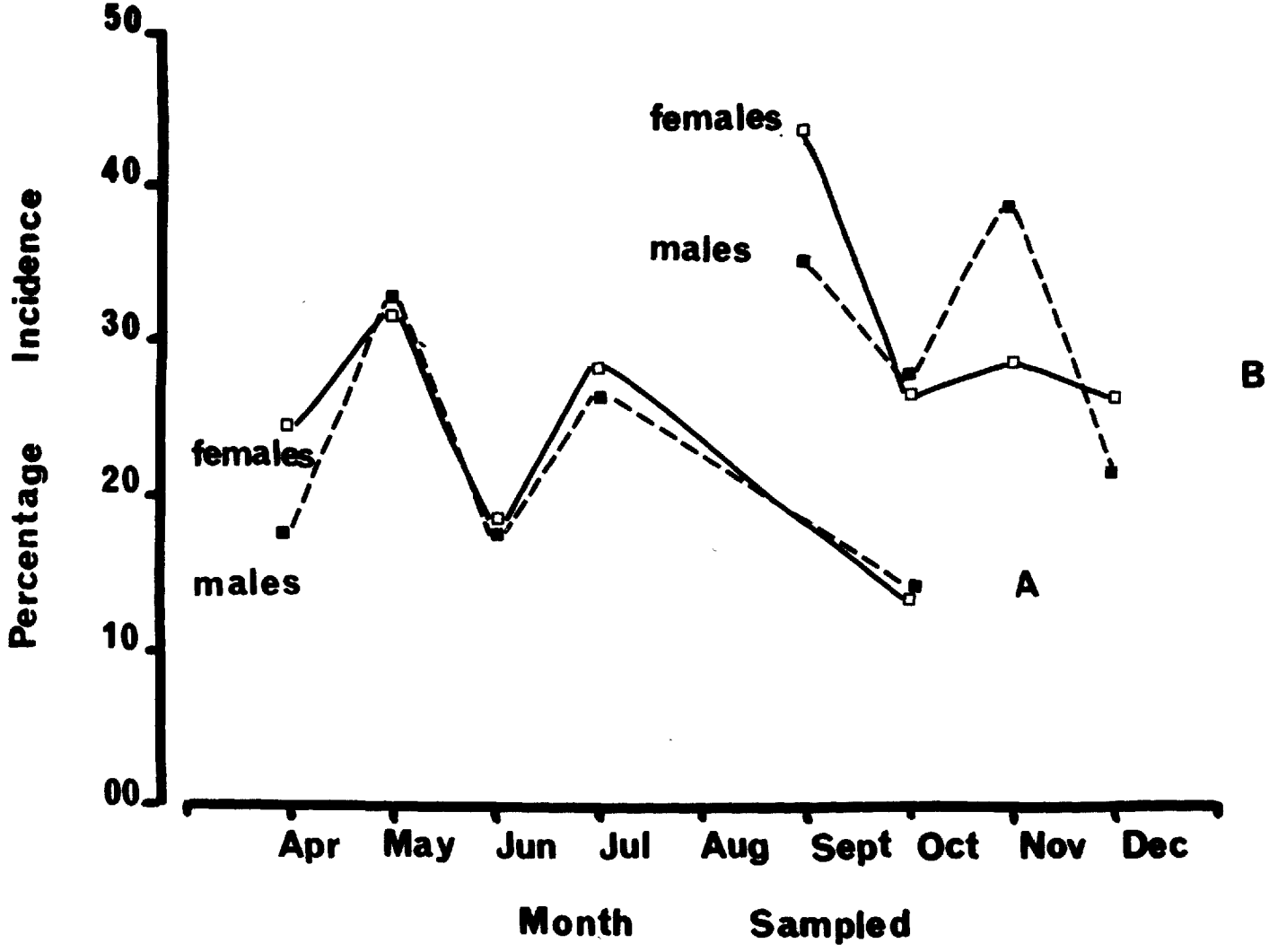
FIG. 13

THE PERCENTAGE PARASITISM OF IPS SEXDENTATUS, BARK BEETLES COLLECTED FROM TWO DIFFERENT LOCALITIES SAMPLED AT MONTHLY INTERVALS.

Key

A. ALICE HOLT

B. YATELY


50
40
30
20
10
00
Percentage Incidence
females
males
females
males
A
B
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Month Sampled

parasites per host was the commonest incidence of parasitism in natural forest populations (Fig. 13). Beetles occurring naturally in pine logs with 20, 30, 40 or up to 60 were rare.

Percentage infestation at different times from different localities are shown (Figs. 13 and 15). The highest infestation was recorded from Yatley in September (35% - 43.5%) and dropped to 25 - 26% in November, while at Alice Holt the highest infestation was 32 - 33% in May, declining to 13 - 14% in October.

The incidence of encapsulated nematodes in adult hosts also varied from time to time, ranging between approximately 5 - 15 percent. Such variations in incidence of encapsulation in adult hosts is presumably related to the age of the beetles, especially in natural forest population where the age of the host was unknown, although only two generations usually emerge in a year (Fig. 14). The first generation appeared in May and June and the percentage encapsulation varied between 16 - 21% and declined in October to 8%. In the second generation which emerged in November, and entered an overwintering diapause, the percentage encapsulation was 15%.

In laboratory cultures the incidence of parasitism

FIG. 14

THE PERCENTAGE INCIDENCE OF ENCAPSULATION IN FOREST POPULATIONS FROM TWO DIFFERENT LOCALITIES

Key

A. ALICE HOLT

B. YATELY

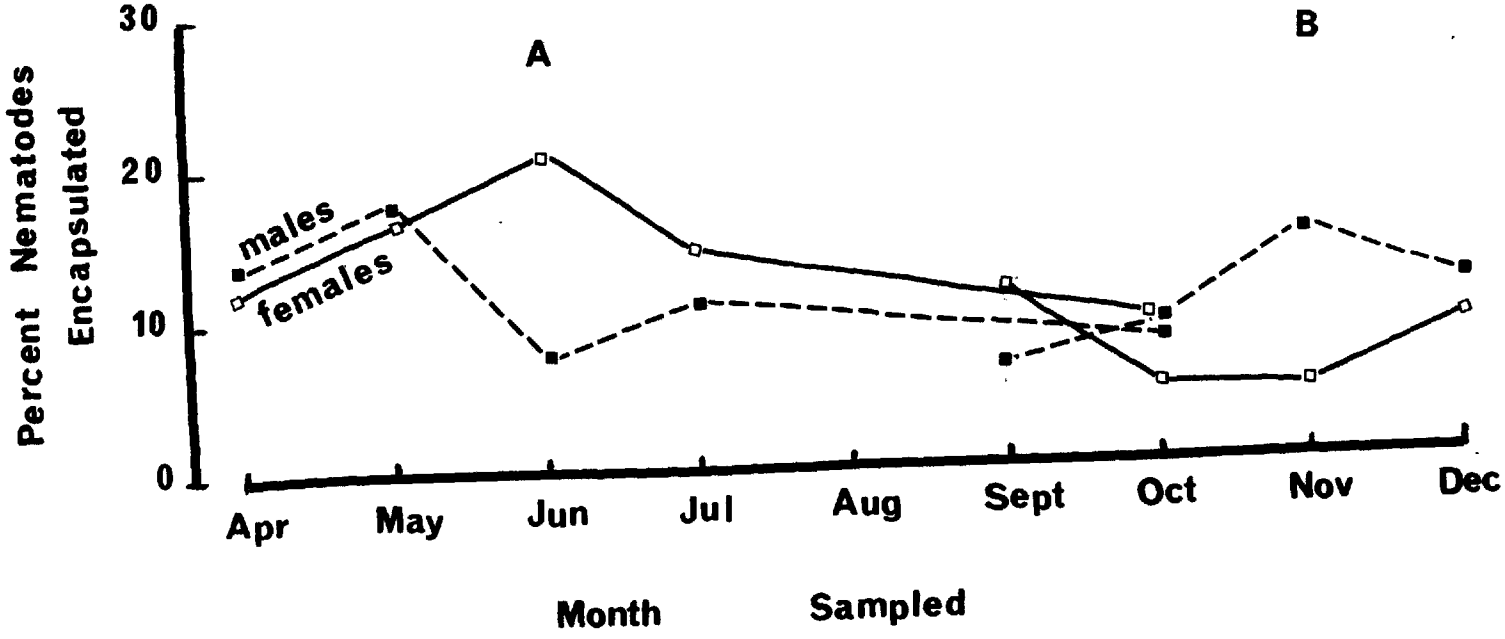

Percent Nematodes Encapsulated
30
20
10
0
A
B
males
females
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Month Sampled

FIG. 15

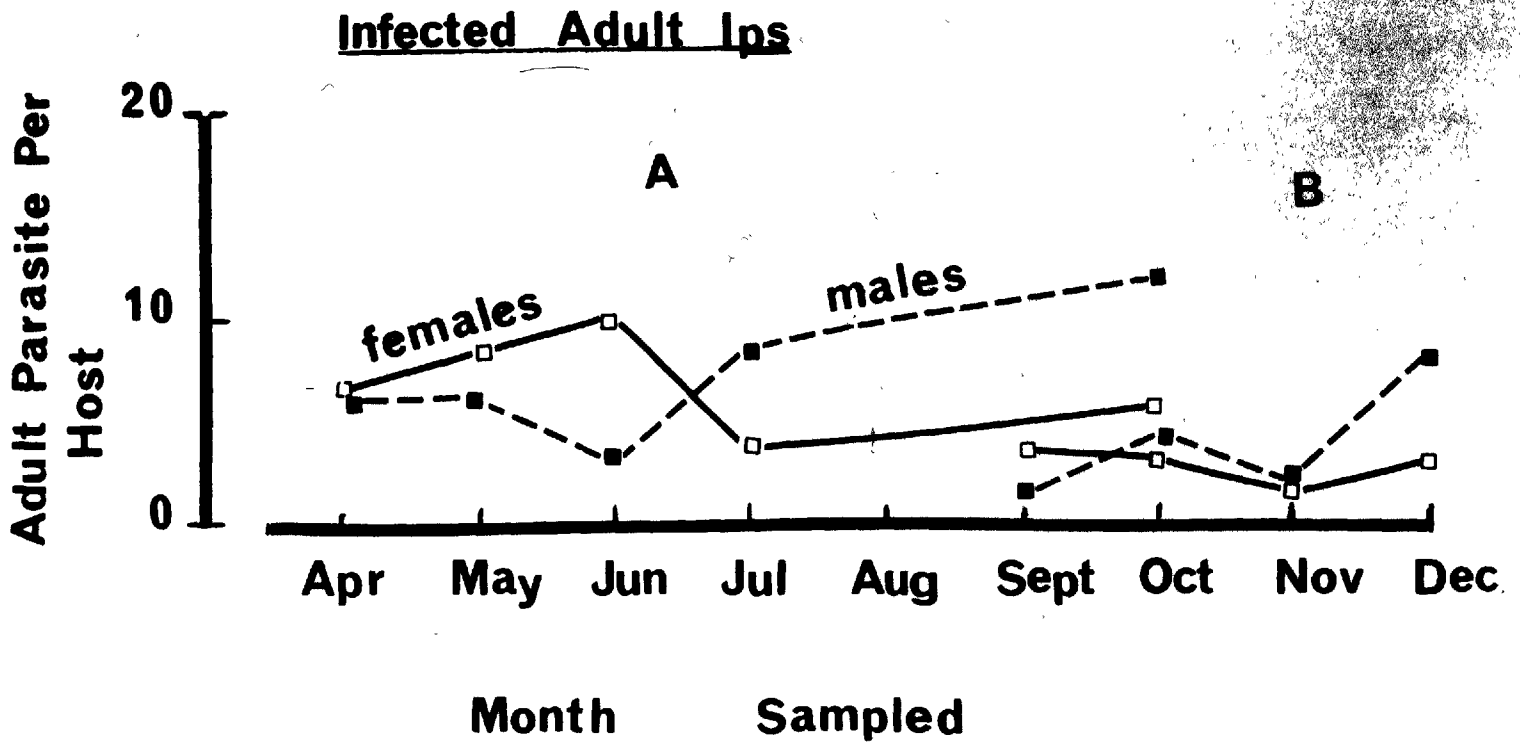
PARASITE DENSITY IN INFECTED ADULT

IPS SEXDENTATUS IN FOREST POPULATIONS

Key

A. ALICE HOLT

B. YATELY


Infected Adult Ips
Adult Parasite Per Host
20
10
0
A
B
females
males
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Month Sampled

varied from generation to generation depending on the initial infestation of the parents. The incidence of encapsulation was directly related to the age of the adult beetle. 75% of parasitism was recorded in the 5th generation but in the last two generations the incidence dropped to 29 - 34%. It is possible that this low incidence was caused by a very low percentage infection in the beetles used in subculturing. 36.8% incidence of encapsulation was also recorded in the 5th generation Table 5a. Number of parasites per host varied between 1 - 150 with few beetles containing a single parasite. The introduction of infested beetles produced a highly infested population, possibly because of the limited distances that the beetles can move.

Table 5a Incidence of parasitism and encapsulation of I. sexdentatus from laboratory cultures

No. of generations	Clone	Total Ips examined	Total Ips infected	Percentage infection	Percentage encapsulation	Range of nematodes of arithmetic means
F1	A	125	33	26.4	15.8	1-26(13.8)
	B	135	32	27.1	18.02	1-34(8.9)
F2	A	125	33	26.4	16.1	1-22(9.4)
	B	122	32	26.2	19.2	1-28(12)
F3	A	130	48	36.9	21.5	1-32(14.6)
	B	72	28	38.8	20.6	1-28(12)
F4	A	71	28	39.1	23.4	1-102 (23.5)
	B	167	79	47.3	28.8	1-36(20.5)
F5	A	127	55	43.3	34.0	3-65(30)
	B	85	64	75.2	36.8	1-150(22)
F6	B	108	37	34.2	13	1-45 (15)
F7	B	135	40	29.6	12.6	2-56 (18)
Mean	-	-	42.4	37.5	21.7	-
Standard Error	-	-	±20.5	±17.8	± 10.2	-

A and B represent the two different localities from which the initial samples were done and from these two original samples the laboratory sub-culturing was made.

3.10. Effect of starvation on heavily parasitized host and subsequently on their including parasites

The result (Table 6) shows that after seven days of starvation the most heavily parasitized beetles died. The number of parasites per host in this group varied between 25 - 40 adult parasitic female nematodes with a mean of 34.10. It was found that the hosts can survive longer when they contained fewer parasites, and those which survived until the end of the experiment were non-infected and were still alive. After 25 days some uninfected beetles died. The nematodes inside the dead host had also died.

Table 6 Effect of starvation of parasitized beetles

No. of parasite per host in each group		MT100	No. of beetles in each group
Range	Mean		
25-40	34.1	7	12
10-18	14.3	10	18
5-8	6.6	15	25
3-5	4.2	20	28
0-1	0.12	25	8
0	0	30	20

Key: MT100 is the time taken to reach 100% mortality.

4. Effect of multiple infections on subsequent development internal anatomy and fecundity of the parasitized host

Both small and large male and female beetles were found to harbour the parasite. There was found to be no decrease in size of parasitized beetles when compared with uninfected ones, except when highly infected. Male beetles infected with 80-100 adult female parasites were found to be relatively smaller than others, but these were rarely found. No effect on external characters, as alterations on the external structures of the host due to the presence of the parasites were observed. Consequently it was not possible to detect if a beetle was parasitized by examining its external anatomy, but behavioural differences were noticed and infected insects appear to be less active than non-parasitized ones. The cuticle of an infected insect was always lighter in colour, being light brown instead of being dark brown compared with non infected insects of the same age, indicating that the nematodes retarded the development of the insects. This phenomenon was very useful in obtaining sufficient infected hosts and parasites for various experiments. Emergence of adult beetles from pupae was retarded by nematode infection. Differentiation and growth of the

host was also affected by parasitism, the time taken for an infected pupae to become adult was always longer, about 7 days compared to 3 days for the completion of pupation of unparasitized hosts. Consequently, infected pupae were the last to emerge from logs, and the length of delay was in proportion to the density of the parasites per pupa. Nevertheless dissection was the only certain method of determining parasitism.

The presence of the nematodes in the body cavity of the host, irrespective of their numbers, diminished the fat reserves and destroyed the fat bodies, and in cases of heavy infestation the fat bodies were non-existant. It was found that in dissections of adult beetles the ovaries of non-infected females were well developed, while infected females of the same age had smaller and undeveloped ovaries, especially in heavily parasitized hosts.

Another internal effect, when the nematode is present in the body cavity of the host in large numbers, particularly when the third stage larvae appear very active, the genital system of the beetle is affected. These nematodes appear to pierce and destroy the peripheral sheath so that the ovaries hang loose in the body cavity. Lastly, when they were heavily parasitized Contortylenchus reduced the

egg output of the female beetles. It was found that when four infected adult male and female beetles were used in subculturing, only 16 adult beetles were produced in the next generation (besides the original four which were still alive at harvesting time). They were all found to be heavily infested, which indicated that the multiple parasite infection diminished beetle fecundity.

4.1. Histochemistry

1. Chemical composition of the capsules

The results from most of the histochemical test for the capsules (Table 7) prove that the capsules are mainly composed of protein.

Table 7

Histochemical tests	Capsules tissue
4.1.1. Proteins	
a. aqueous bromophenol blue (general protein)	++++
b. Millon's reaction (structural protein, tyrosine)	++
c. Nucleic acids Feulgen stain (Deoxyribonucleic acid)	+++
4.1.2. Carbohydrates	
a. Best's Carmine (glycogen)	++
b. P.A.S. glycogen and mucopolysaccharides	++
4.1.3. Lipids	
a. Sudan black B (bound lipid)	-
b. Oil red O (neutral lipid)	-

Key: ++++, +++, ++, + — Intensity of positive result

\- — negative

4.1.4. All techniques used to determine the presence of enzymes were negative.

4.1.5. Capsules gave a slight positive reaction for haemoglobin.

4.1.6. Amino acid analysis of the capsules in shown in Table 8. Surprisingly tyrosine was not detected by this method, although the capsule was positive with Millon's reactions which is regarded as specific for tyrosine.

Table 8 Approximate quantities of amino acids in the cellular capsule

Amino Acids detected		Quantity of each amino acid in the capsule in nanamoles/ml	Percentage of total amino acids present
1	Glutamic acid	53.0	9.5
2	NH_3	50.1	9.2
3	Glycine	50.0	8.9
4	non lucine	50.0	8.9
5	isolucine	50.0	8.9
6	Aspartic acid	46.8	8.3
7	Lucine	42.3	7.5
8	Serine	41.9	7.4
9	Lysine	38.5	6.8
10	Alanine	36.3	8.6
11	Threonine	23.5	4.2
12	Argnine	20.1	3.5
13	Phenylalanine	16.1	2.8
14	Valine	15.5	3.1
15	Methionine	4.54	0.8
16	Butidine	2.38	0.4
17	Methionine sulphate	1.5	0.2
18	Butyric acid	1.11	0.1

Chemical composition of adult parasitic female nematodes

The cuticle of the female nematodes was slightly positive to all the lipid stains used an a small amount of bound lipid (Sudan Black B) was detected at the base. The cuticle was positive for mucopolysaccharides (PAS) and slightly for glycogen which appeared as a fine granular material (Best carmine), but it was strongly positive for general proteins (bromophenol blue) and inconclusive with Millon's reaction. None of the enzyme techniques used demonstrated activity except for very slight indication acid phosphatase.

The hypodermis contained neutral fat (oil red O) and bound lipid (Sudan Black B) present as granules, carbohydrates were strongly positive (Best carmine) and it was also positive for PAS: but it was strongly positive for general proteins (bromophenol blue). The hypodermis cell nuclei stained intensly for DNA (Feulgen). The hypodermis showed slight acid phosphatase activity, but this was inconclusive.

The coelomocytes in the pseudocoelom stained strongly positive for mucopolysaccharides and glycogen (PAS). The oesophageal region contains lipid droplets (oil red O), proteins (bromophenol blue), mucopolysaccharides (PAS) and glycogen (Best carmine). The lumen of the oesophagus

stained strongly positive for mucopolysaccharide and protein (PAS, Bromophenol blue). The nuclei in this region stained strongly for DNA (Feulgen). The gut of the adult female filled with neutral fat, weakly positive for PAS and Best carmine and positive for proteins. The gonads contained large amount of lipid, glycogen and mucopolysaccharides (Sudan black B, bromophenol blue Best carmine and PAS), the ovary and the eggs and the oviduct were intensly positive for proteins (bromophenol blue) and for DNA (Feulgen) especially the eggs.

Positive reactions were obtained for the haemoglobin test, but it was not very satisfactory, it is demonstrated in the cuticle the gut and the gonads, but the results were inconclusive, it needs more certainty for adequate controls were not used.

4.2. Osmotic regulation of unencapsulated nematodes and entire capsule

The effect of immersing adult female Contortylenchus in various dilutions of 1.0M NaCl, 1.0M sucrose and different dilutions of entomological saline are shown in Figs 16 to 19. It was found that the length of the nematode was affected by the concentration of the solutions used. After 90 - 120 minutes in hypertonic solution there was no more shrinkage and after 360 minutes in 0.6M NaCl the nematodes had regained the original length and appearance (Fig. 16). It took the nematodes in 0.4M and 0.5M NaCl up to 15 hrs. to regain their original length. Nematodes in 0.4M, 0.5M and 0.6M NaCl were still alive after 15 hrs., whilst those in 0.8M and 1.0M NaCl returned to their original length but were all found to be dead after 20 - 22 hrs.

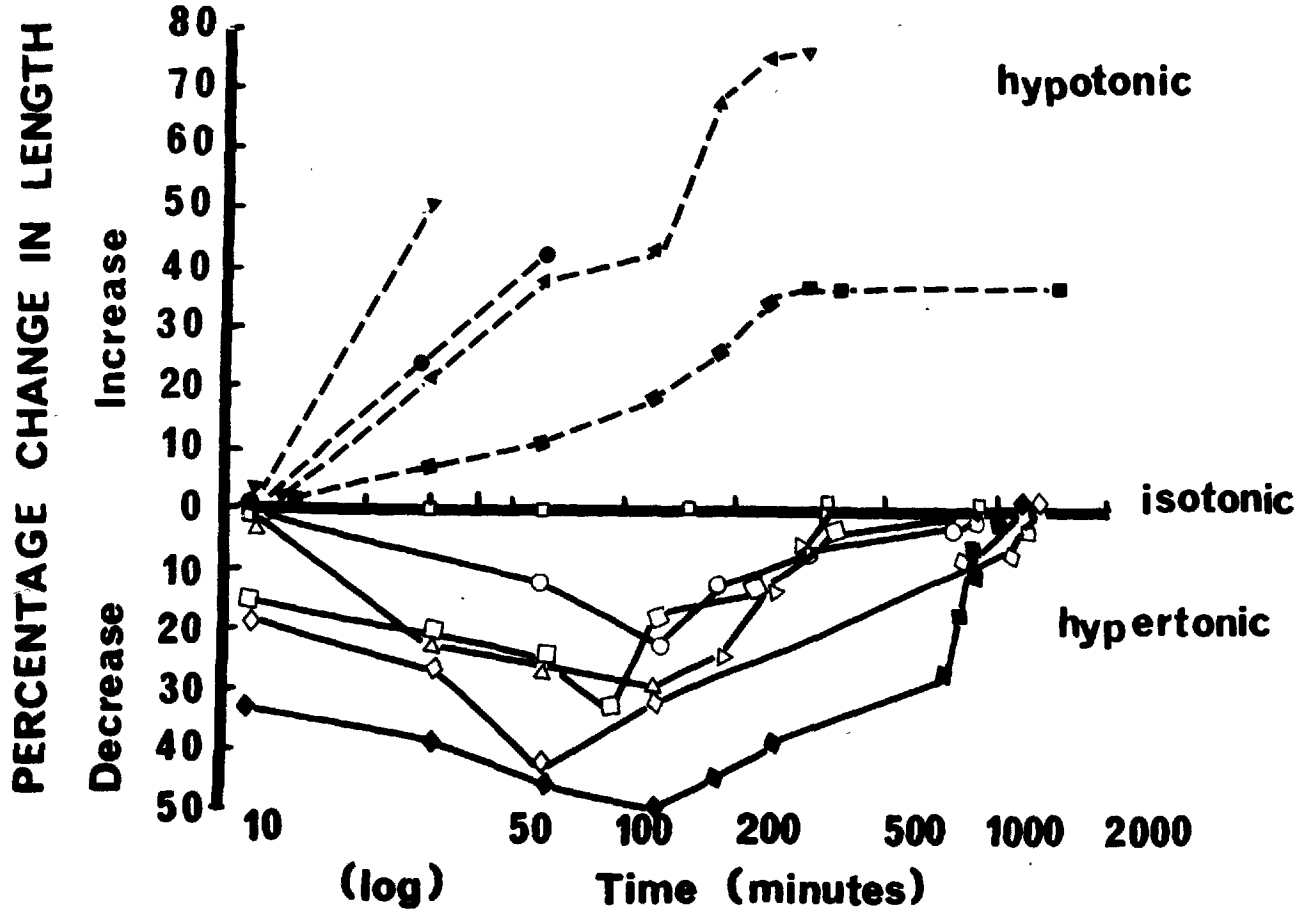
In distilled water or 0.05M, 0.10M and 0.20M NaCl the nematodes were unable to regain their original length after 30-300 min. In distilled water, 0.05M and 0.10M NaCl they all burst, while in 0.2M they had not regained their original length within 24 hrs. even though they were still alive. No change in length occurred in 0.3M NaCl, which was taken to be isotonic (Fig. 16). Similar results were obtained when the

FIG. 16

THE EFFECT OF VARIATIONS IN OSMOTIC PRESSURE UPON ADULT PARASITIC FEMALES CONTORTYLENCHUS DIPLOGASTER NEMATODES (MEAN OF FIVE) WITH TIME PLOTTED ON LOG SCALE IN DISTILLED WATER AND VARIOUS CONCENTRATION OF SODIUM CHLORIDE

Key

▲-▲	.	Dis. H_2O	
●-●	.	0.05 M	Sodium Chloride
▼-▼	.	0.1 M	" "
■-■	.	0.2 M	" "
□-□	.	0.3 M	" "
○-○	.	0.4 M	" "
△-△	.	0.5 M	" "
□-□	.	0.6 M	" "
◇-◇	.	0.8 M	" "
◆-◆	.	1.0 M	" "


PERCENTAGE CHANGE IN LENGTH
Increase
Decrease
80
70
60
50
40
30
20
10
0
10
20
30
40
50
hypotonic
isotonic
hypertonic
10
50
100
200
500
1000
2000
(log)
Time (minutes)

nematodes were placed in hypertonic solutions of entomological saline they regained their original length after a period of shrinkage, while there was no recovery in hypotonic solutions except at 0.85%. It was found that they could survive longer in 0.85% entomological saline although they increased in length at the beginning, but began to recover and after 90 min. they regained their original length. This was the only instant of apparent recovery in a hypotonic solution. They did not burst even after 24 hrs. (Fig. 17).

Nematodes placed in hypertonic sucrose solutions continued to shrink for more than 3 hrs (Fig. 18), then a reduction in the rate of shrinkage occurred but they were unable to regain their original length even after 18 - 20 hours, but they approached their original length after 2 hrs. The nematodes increased in length in hypotonic solutions of sucrose (0.2M) for 18 hours.

Those which had regained their original length in hypertonic solutions of NaCl after 15 hrs. or less and were placed in 0.3M NaCl increased in length as if they were in hypotonic solutions (Fig. 19).

The effect of immersing capsules in various dilutions of 1.0M NaCl and different dilutions of sea water are shown in Fig. 20. It was found that the extent of change

FIG. 17

SHOWING THE PERCENTAGE INCREASE (+) OR DECREASE (-) IN LENGTH OF ADULT PARASITIC FEMALES CONTORTYLENCHUS DIPLOGASTER (MEAN OF FIVE) WITH TIME PLOTTED ON A LOG SCALE IN VARIOUS DILUTIONS OF ENTOMOLOGICAL SALINE

Key

●·●· ·	0.90%	Entomological Saline
○·○· ·	0.85%	" "
□·□· ·	0.80%	" "
■·■· ·	0.75%	" "

Mean Pecentage Change
In Length
40
30
20
10
0
10
20
decrease
increase
10
50
100
200
500
1000
2000
(log)
time (minutes)

FIG. 18

PERCENTAGE DECREASE (-) OR INCREASE (+) IN LENGTH OF C. DIPLOGASTER IN DIFFERENT MOLARITIES OF SUCROSE (MEAN OF FIVE) WITH TIME PLOTTED ON A LOG SCALE

Key

●.●. . 0.2 M Sucrose

▲.▲. . 0.4 M "

△.△. . 0.6 M "

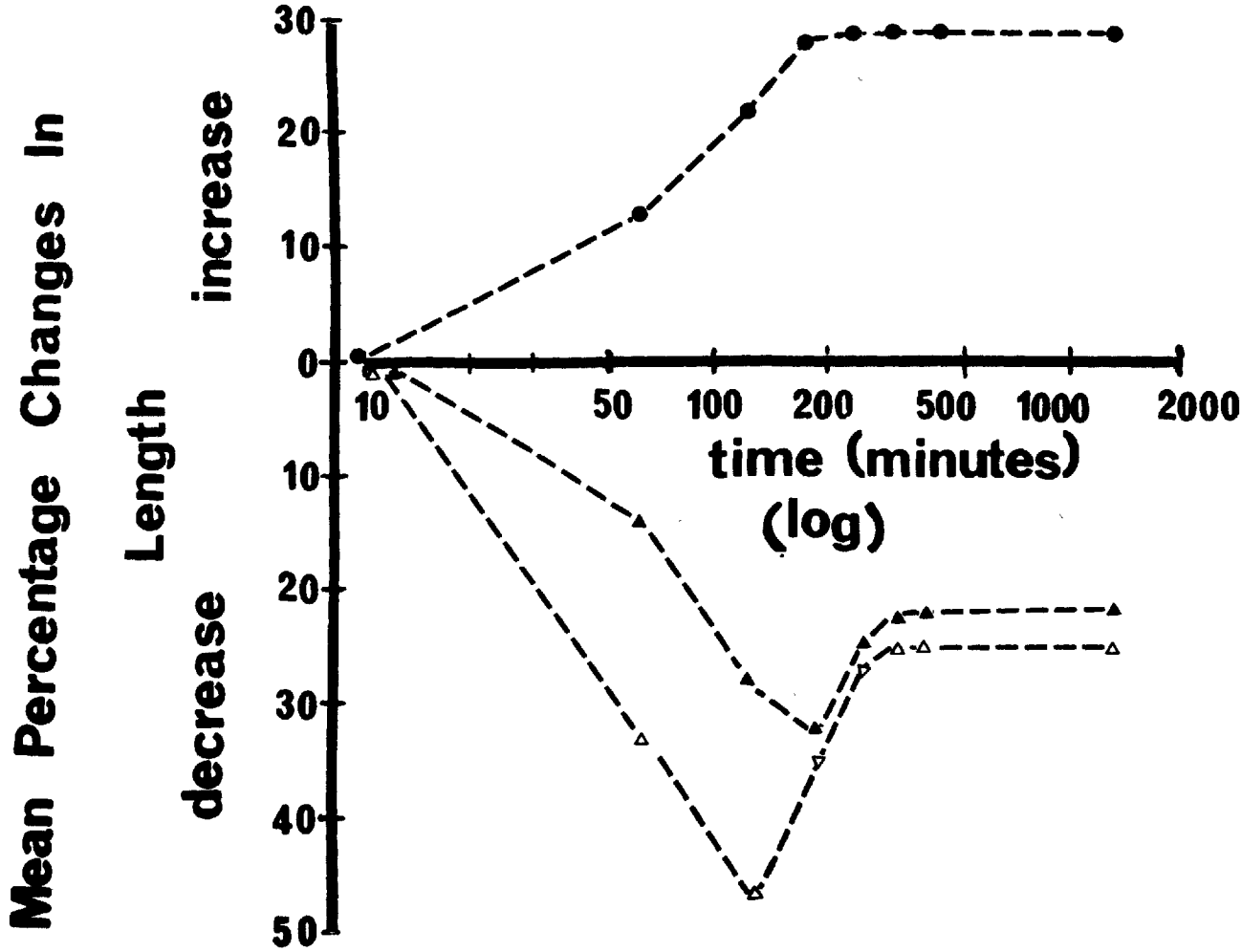

Mean Percentage Changes In Length
increase
decrease
30
20
10
0
10
20
30
40
50
10
50
100
200
500
1000
2000
time (minutes)
(log)

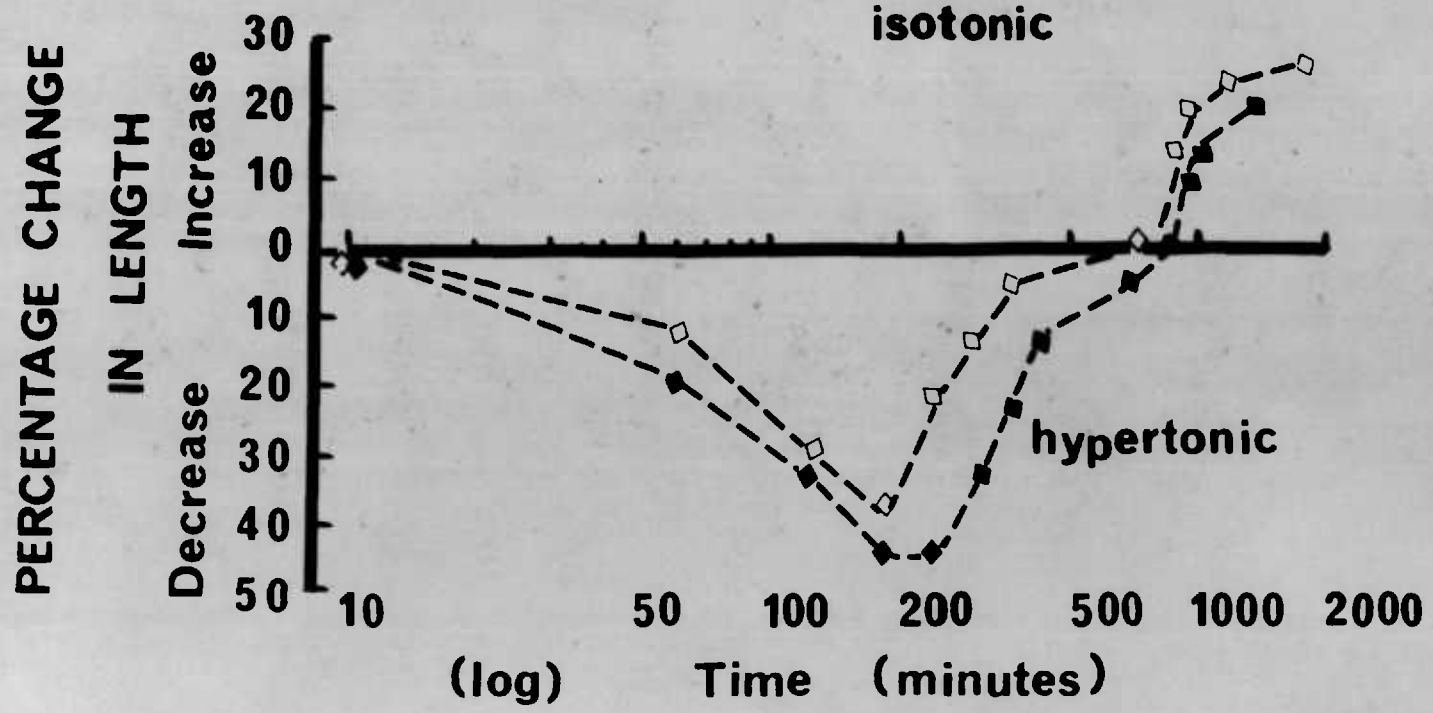
FIG. 19

SHOWING THE PERCENTAGE DECREASE IN LENGTH OF C. DIPLOGASTER IN 0.8 M AND 0.6 M SODIUM CHLORIDE, THEN INCREASE IN LENGTH AFTER BEING IN ISOTONIC SOLUTION 0.3 M SODIUM CHLORIDE, (MEAN OF FIVE) WITH TIME PLOTTED ON A LOG SCALE

Key

◇·◇· . 0.6 M Sodium Chloride

◆·◆· . 0.8 M " "


PERCENTAGE CHANGE
IN LENGTH
Increase
Decrease
30
20
10
0
10
20
30
40
50
isotonic
hypertonic
10
50
100
200
500
1000
2000
(log)
Time (minutes)

in the size of the capsules was effected, firstly, by the strength of the solution and secondly, by the nematodes inside that capsule. When the solution was hypotonic for the capsule it was hypertonic for the nematode inside it. In 0.75M NaCl the capsule began to expand at the beginning and the nematodes shrank, when the nematodes began to regain their original length, the capsule was reduced in size, so there was no equilibrium in the changes. It was impossible to run the experiments more than 18 - 22 hrs., because after that the nematodes died and the capsules became dull and cloudy, so making observation difficult, but no stable equilibrium had apparently been reached. Less change occurred in 1.35M NaCl. The capsule was reduced in size and expanded little after 60 min. and no more after 8 hrs. Greater expansion occurred in 1.0 and 1.25M NaCl and greater reduction in 1.5M NaCl. Capsules placed in different dilutions of sea water were more stable. 80% (0.4 M NaCl) sea water was hypertonic to the capsules and caused greater reduction in size of the capsules after 30 min., but the capsules regained their original size after 8 hrs. They began to expand, possibly because of the nematodes inside but there was less than 5% expansion. The experiment stopped after the death of the nematodes. Capsules placed in 70-75%

FIG. 20

PERCENTAGE CHANGES IN WEIGHT OF THE CAPSULE IN DIFFERENT MOLARITIES OF SODIUM CHLORIDE (MEAN OF FOUR) WITH TIME PLOTTED ON A LOG SCALE

Key

O•O	.	1.5 M Sodium Chloride
●•●	.	1.35 M " "
□•□	.	In 0.75 M first then in 1.5 M Sodium Chloride
△•△	.	1.0 M Sodium Chloride
■•■	.	1.25 M " "
▲•▲	.	0.8 M " "

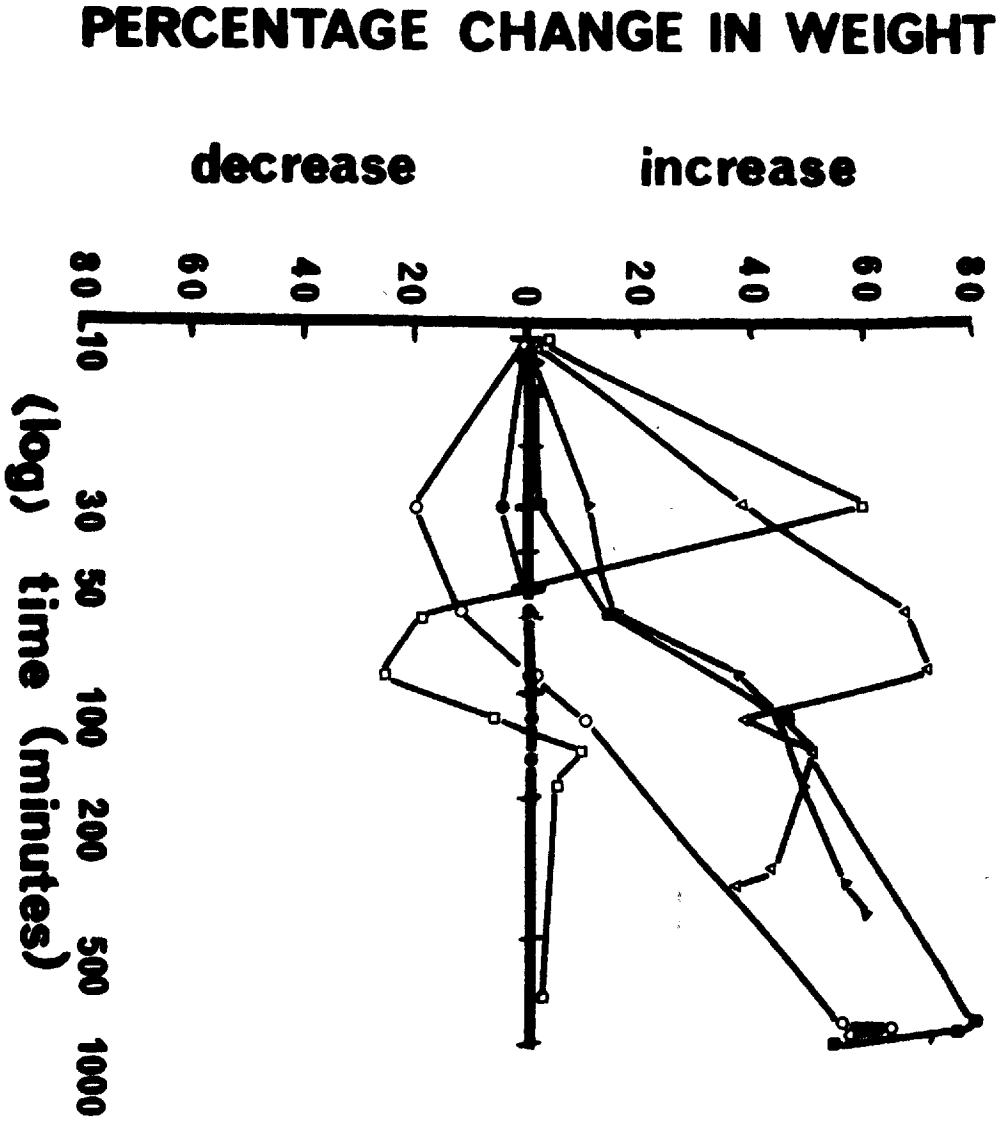

PERCENTAGE CHANGE IN WEIGHT
decrease
increase
80
60
40
20
0
20
40
60
80
10
30
50
100
200
500
1000
(log) time (minutes)

FIG. 21

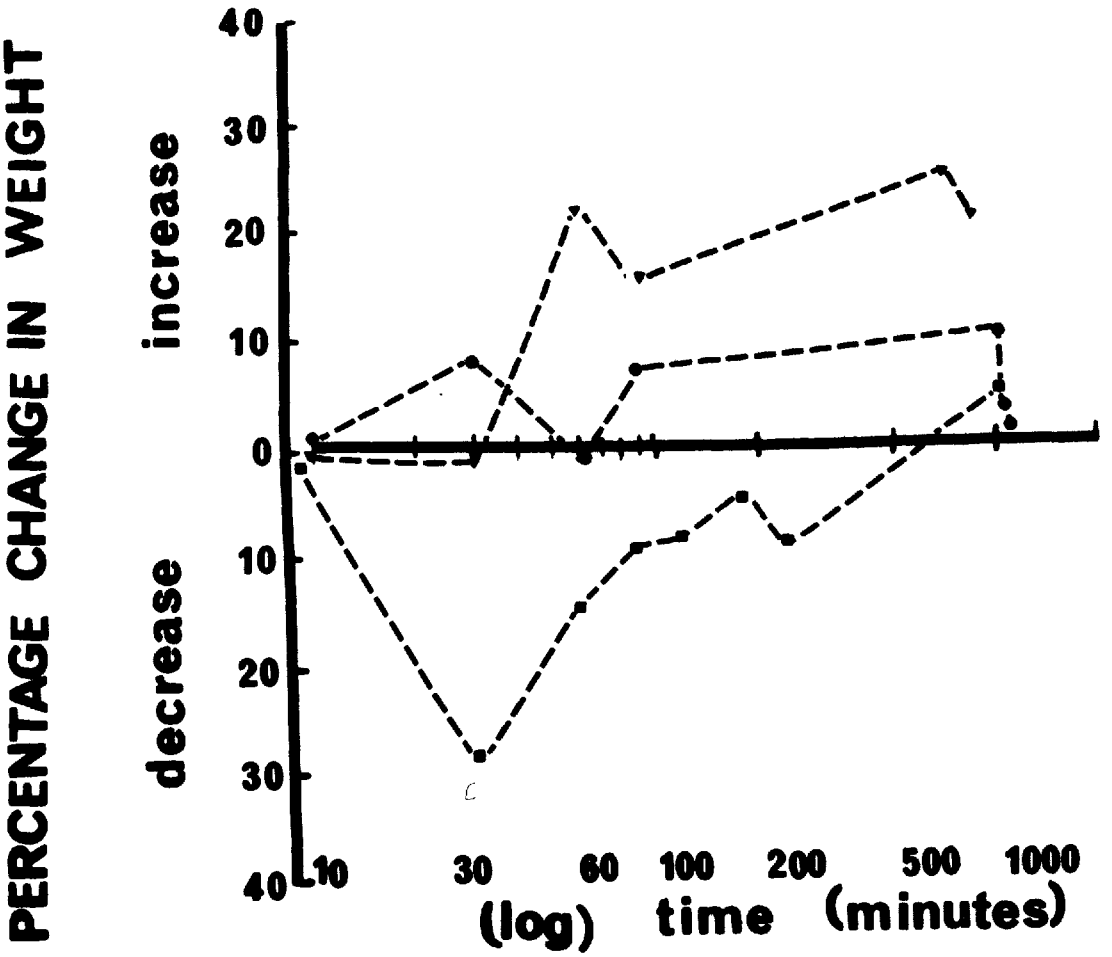
PERCENTAGE CHANGES IN WEIGHT OF THE CAPSULE IN VARIOUS DILUTIONS OF SEA WATER (MEAN OF FOUR) WITH TIME PLOTTED ON A LOG SCALE

Key

▲-▲ 70% Sea water

●-● 75% " "

■-■ 80% " "


PERCENTAGE CHANGE IN WEIGHT
increase
decrease
40
30
20
10
0
10
20
30
40
10
30
60
100
200
500
1000
(log) time (minutes)

sea water increased in size so these dilutions were hypotonic to the capsules, there was very little recovery so these dilutions were considered to be hypotonic to the capsules. There was very little recovery in these solutions but they were not able to regain their original size even after 15 - 17 hours, (Figs. 20 and 21).

Continued on page 204

Plates 30 A, B and C showed the capsules exposed to different concentrations of solutions. In A it shows the capsule in a hypotonic solution where it expanded but the nematodes appeared as if they were in hypertonic solution. While in B the capsule and the nematode are in hypotonic solutions where both appeared swollen and the capsule nuclei appeared clearly. The capsule and the nematode appeared shrunken in Plate 30 C which means they are exposed to hypertonic solution.

PLATE 30

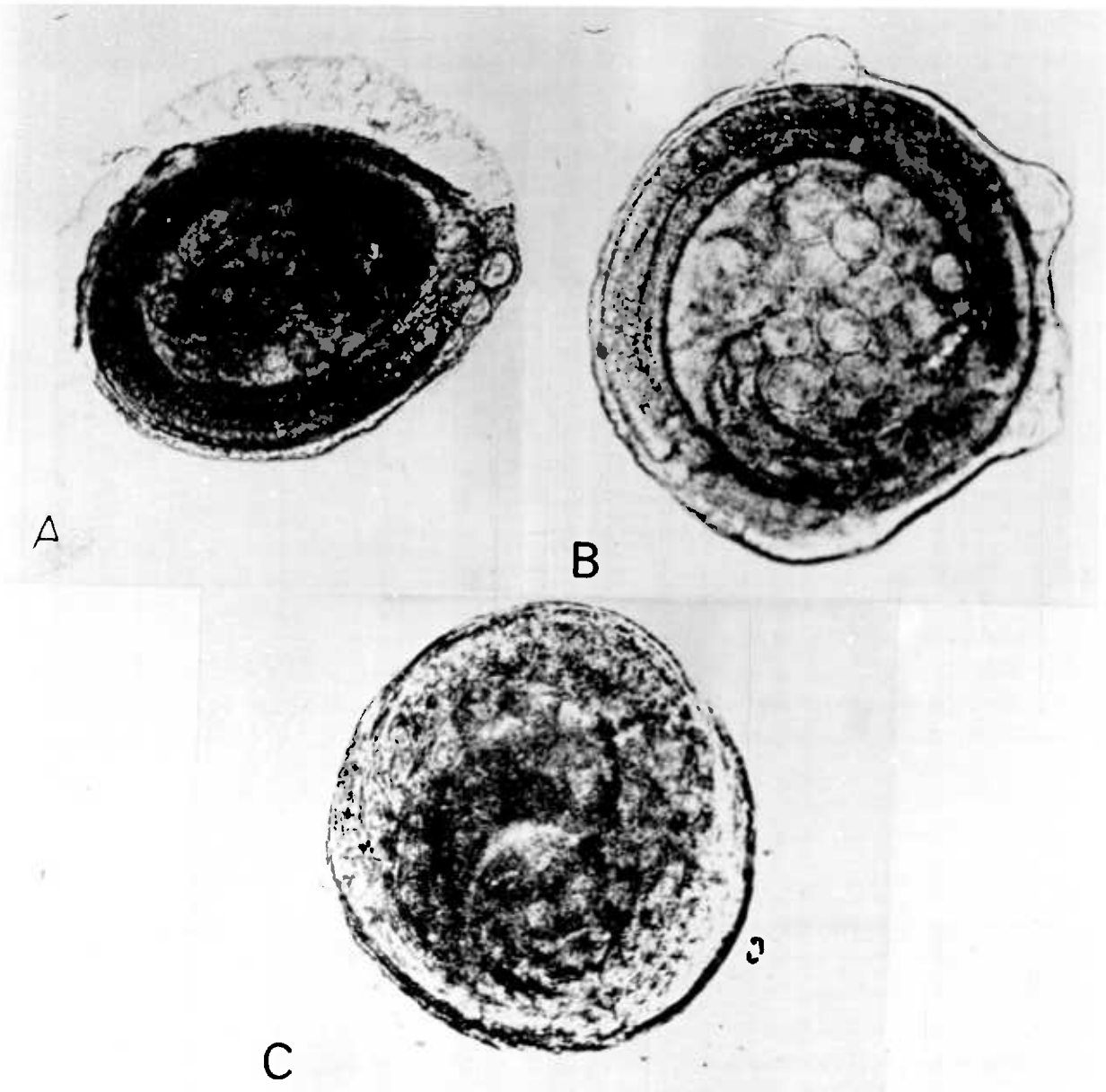
THE EFFECT OF VARIATION IN OSMOTIC PRESSURE UPON THE CAPSULE OF C. DIPLOGASTER

A. CAPSULE IN HYPOTONIC SOLUTION BUT THE NEMATODE IN HYPERTONIC

B. THE CAPSULE AND THE NEMATODE IN HYPOTONIC SOLUTION

C. THE CAPSULE AND THE NEMATODE IN HYPERTONIC SOLUTION

(X 100)


A
B
C

4.3. The oxygen consumption of infected and uninfected adult beetles

The respiration rate was measured for uninfected male and female adult beetles.

The mean respiration rate was almost the same in uninfected male and female beetles (Table 9) although there was a considerable variation in oxygen consumption of beetles of the same sex. These results were compared with the consumption rates obtained for infected beetles, in order to determine if the O_2 consumption rate was affected by the presence of the parasites. It was found that the parasitized beetles had a higher rate than uninfected beetles (Fig. 22).

Table 9 Oxygen consumption (QO_2) rate in $\mu l O_2/\mu g$ d.wt./hr. (at 24.4°C) of uninfected adult male and female beetles

Sex and condtion of beetles	No. of replic-ates	Mean $ul O_2$ µg dry wt/ 1 hr.	Range oxygen consumed	Standard error (S.E.)
Uninfected male adult beetles	4	4.699	4.04-5.51	0.349
Uninfected female adult beetles	5	4.818	3.86-5.90	0.399

FIG. 22

THE EFFECT OF PARASITE DENSITY ON THE OXYGEN CONSUMPTION RATE OF IPS SEXDENTATUS PARASITISED BY CONTORTYLENCHUS DIPLOGASTER NEMATODES

Key

Δ•Δ equation points

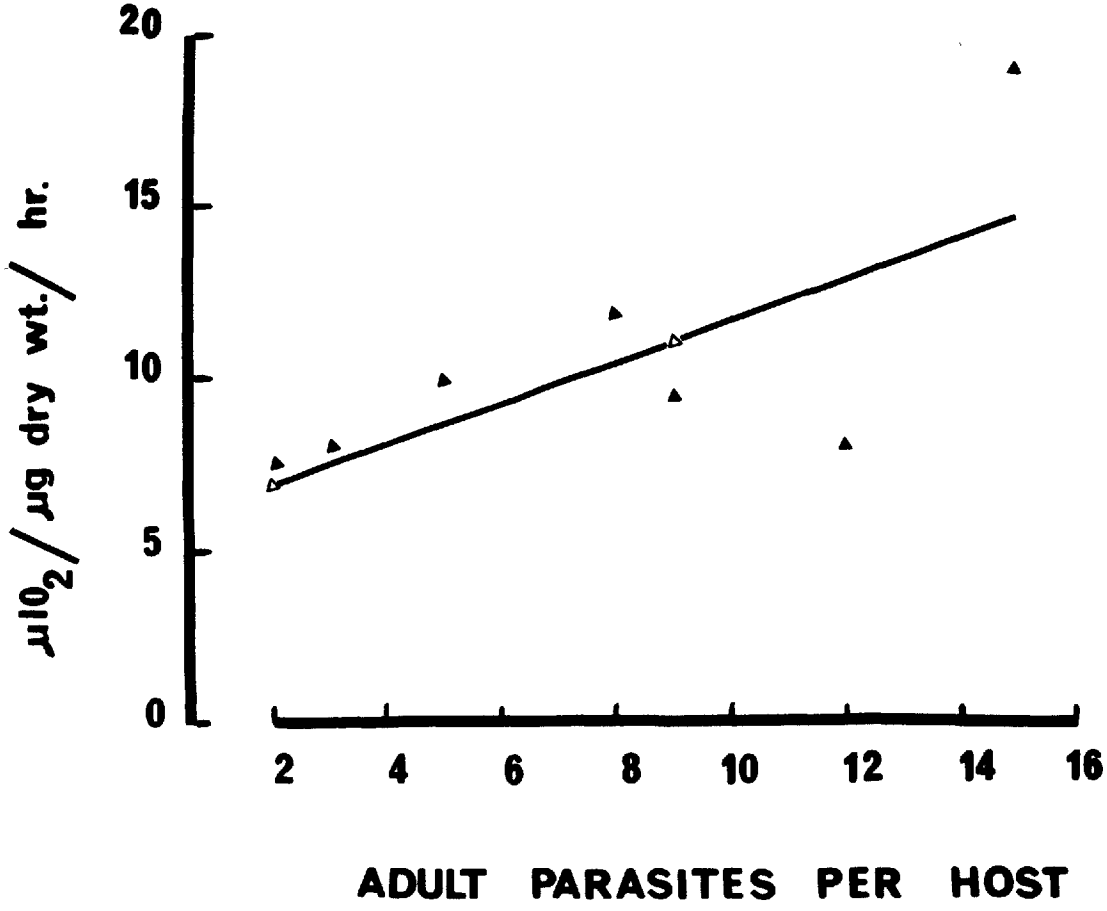

20
15
10
5
0
$\mu l O_2 / \mu g$ dry wt./ hr.
2
4
6
8
10
12
14
16
ADULT PARASITES PER HOST

Table 10 Oxygen consumption rate (at 24.4°C) in $\mu l O_2/\mu g/hr$ of parasitized male and female adult beetles

Sex and condition of beetles	No. of parasites per host	No. of replicates	Mean $\mu l O_2$/ µg dry wt/hr.
Infected adult male beetles	3	2	9.215
	5	2	9.435
	15	1	22.42
Infected adult female beetles	3	2	6.632
	5	2	7.745
	15	1	13.245

It was found that the respiration rate of parasitized beetles was proportional to the parasite density (Fig. 22). The rate was increased steadily as the number of parasites present per individual host increased. Student's "t" test gave significant value for 5 degrees of freedom P = 0.01, since the value of "t" was greater than the observed value at 5 degrees of freedom for 10% level which is 2.015 . Infected male beetles tend to have a higher oxygen consumption rate than infected females (Table 10).

4.4. Respiration rates of adult parasitic female Contortylenchus sp.

The results of the experiments at 30°C showed that

the adult parasitic female Cortortylenchus dissected from hosts had a high oxygen consumption rate, with the mean of 0.477 ± 0.0475 $\mu l O_2/\mu g$ dry weight/1 hr. at 30°C. There was a little variation in the rates, ranging between 0.347 - 0.573 $\mu l O_2/\mu g$ dry weight/1 hr. (See appendix 4).

4.5. The ultrastructure of the young and the mature parasitic adult female nematodes from pupal and adult hosts

The body wall of parasitic larvae of Contortylenchus is composed of an outer layer cortex (Plate 41) with a triple layered structure, two electron dense regions separated by an electron lucid region. The parasitic larval cuticle was ridged transversely at regular intervals. Under the triple-layered membrane there was an amorphous dense layer followed by another narrower dense layer.

In a transverse section the mature parasitic female of Contortylenchus did not appear like other nematodes in the distribution of the muscles or the hypodermis. The body wall was unlike the usual nematode cuticle. The hypodermis was continuous with the outside covering layer. It also had an incomplete layer of fibres which had an irregular region of concentric fibres.

Five layers can be distinguished between the external surface and the muscular region. All these layers form a region 0.93 µm wide. This electron dense region is followed by a granular layer, then a fibrillar layer, a vacuolated region with a column of dense fibres and lastly by the basal lamina which is a band of fine

granular material (Plates 33, 34, 35 A & B, and 36).

The external surface of the adult parasitic female is covered with a membranous layer (Plates 34B, 35, 36, 37, 38 A, C, D, 39A, B and 40). It was impossible to detect this membranous covering layer with the light microscope. These membranes were developed only in the adult parasitic female from the adult host beetle (plate 40, 1, 2, and 3). This membrane appeared as dense granules accummulated on the surface of the nematode. At the same time the infolding of the surface layer started to form here (Plates 33 A, 34A).

In transverse section from young adult parasitic females at the ultrastructural level, the hypertrophy of the hypodermis, particularly in the intercordal regions, was distinct. The reduction of the somatic musculature was extreme. It was apparent that a marked reorganisation and obvious modification in the body wall had taken place after the infected nematode had parasitised the host. (Plates 31 and 32). The hypodermis contained large nuclei with their nucleoli, mitochondria, glycogen and ribosomes. The body of a young parasitic female from host pupa showed the hypertrophy of the hypodermis in the anterior region (Plates 31 and 32).

The somatic musculature is composed of four sectors

PLATE 31

A. TRANSVERSE SECTION THROUGH THE DEVELOPING PARASITIC CONTORTYLENCHUS FROM HOST PUPA IN THE ANTERIOR REGION

(X 100320)

Key

(a, b, and c, the three possible places for fold formation)

ca	canaliculi arrow
ct	capsule tissue
d	duct
f	fibrillar layer
gl	dense glycogen granules
H	lateral hypodermal cord
h	hypodermis
hc	hypodermal cell
hn	hypodermal nucleus
m	mitochondria
mg	mucopolysaccharide granules
[illegible]	
mu	muscles block
no	nucleolus
nu	nucleus
sf	fold of the surface layer
ve	vesicle
p	pseudocoelom

PLATE 31 A

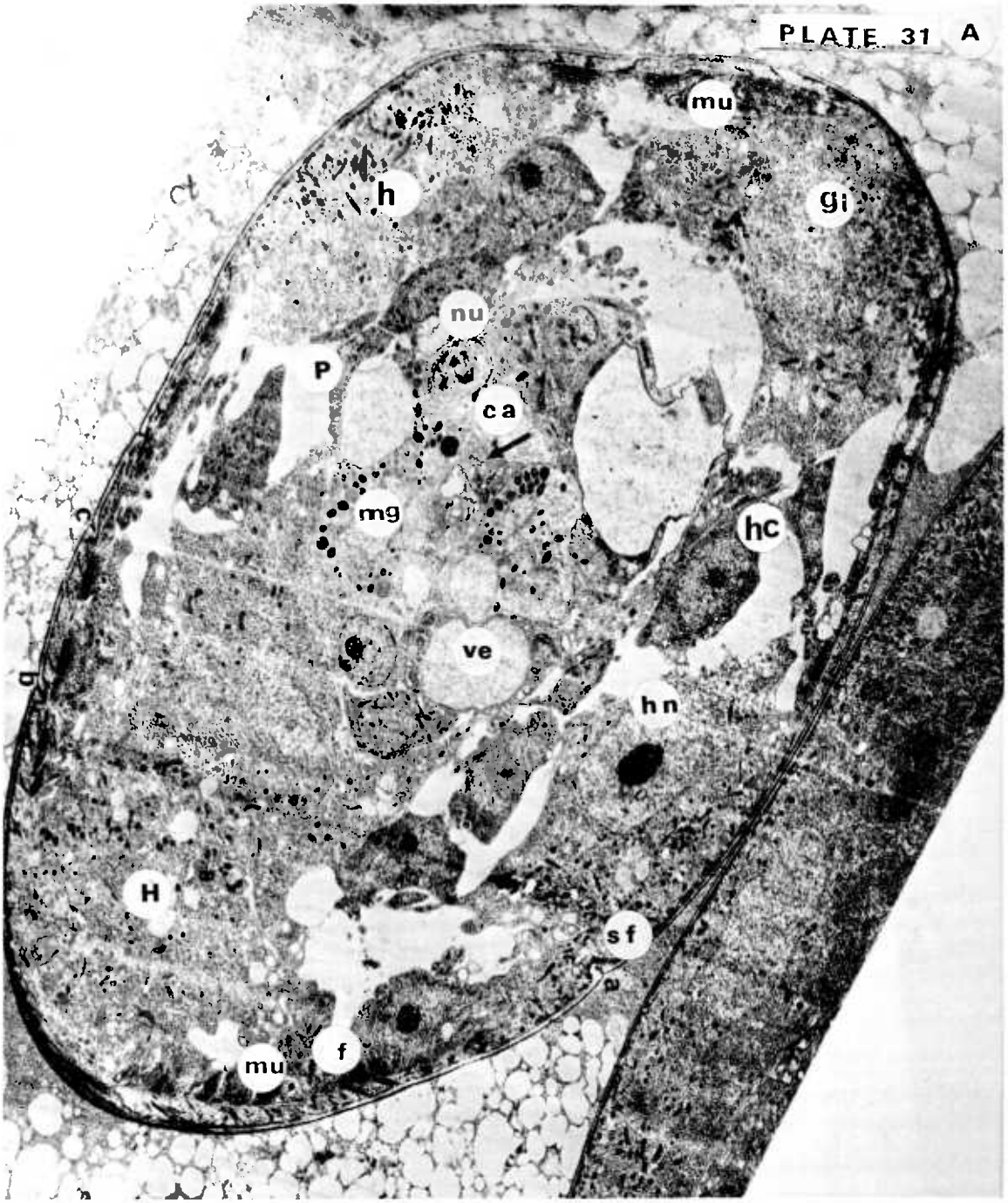


PLATE 31

B. THE CENTRAL REGION MAGNIFIED

C. SECRETORY REGION MAGNIFIED

B. (X 6384) C (X 25080) inset (X 6382)

Key

ca	canaliculi
d	duct
gl	dense glycogen granules
h	hypodermis
hc	hypodermal cell
hn	hypodermal nucleus
m	mitochondria
mg	mucopolysaccharide granules
mt	microtubules
no	nucleolus
nu	nucleus
ve	vesicle
p	pseudocoelom

31 B and C

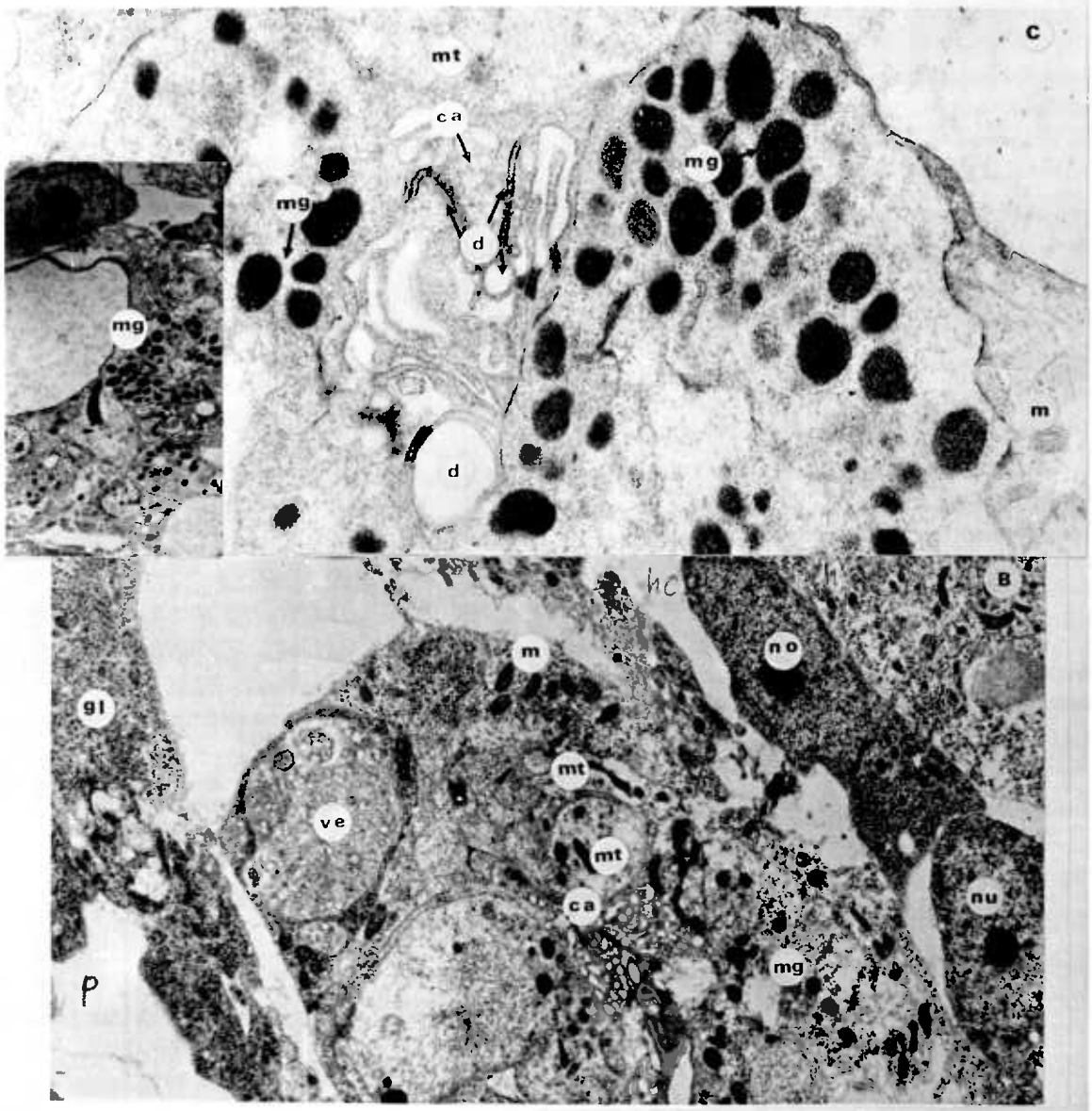


PLATE 32

TRANSVERSE SECTION THROUGH THE DEVELOPING PARASITIC FEMALE CONTORTYLENCHUS IN THE ANTERIOR REGION

The inset (the central region magnified)

Key

ca	canaliculi
ct	capsule tissue
d	duct
gb	golgi body
gl	glycogen
H	lateral hypodermal cord
h	hypodermis
l	lipid
mu	somatic muscle cell
mt	mitochondria
nuc	capsule nucleus
nu	nucleus
ve	vesicle

(X 6384) inset (X 9120)

PLATE 32

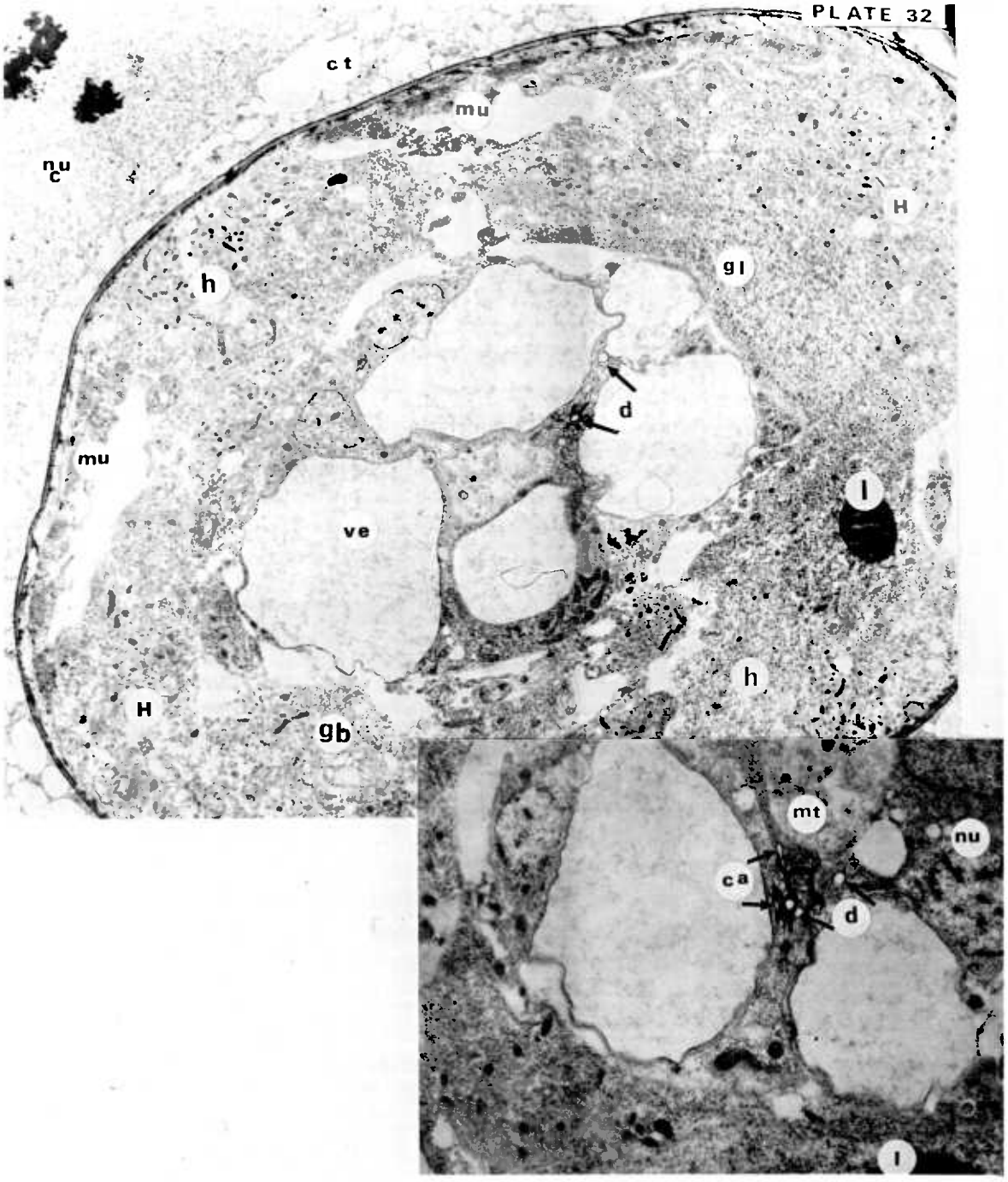


PLATE 33

A. OBLIQUE LONGITUDINAL SECTION OF A DEVELOPING PARASITIC FEMALE OF CONTORTYLENCHUS FROM HOST PUPA

B. LONGITUDINAL SECTION THROUGH THE ANTERIOR CONE OF A DEVELOPING PARASITIC FEMALE CONTORTYLENCHUS

C. LONGITUDINAL SECTION THROUGH THE BODY WALL IN THE LATERAL HYPODERMAL CORD OF ADULT PARASITIC FEMALE CONTORTYLENCHUS

Key

aco	anterior cone
ct	capsule tissue
g	dense granules on the nematode surface
gl	glycogen
H	lateral hypodermal cord
h	hypodermis
l	lipid
mn	muscular tissues
p	pseudocoelom
s	stylet
sf	fold of the surface layer
f	fibrillar layer

A. (X 20520) B. (X 9120) C. (X 4256)

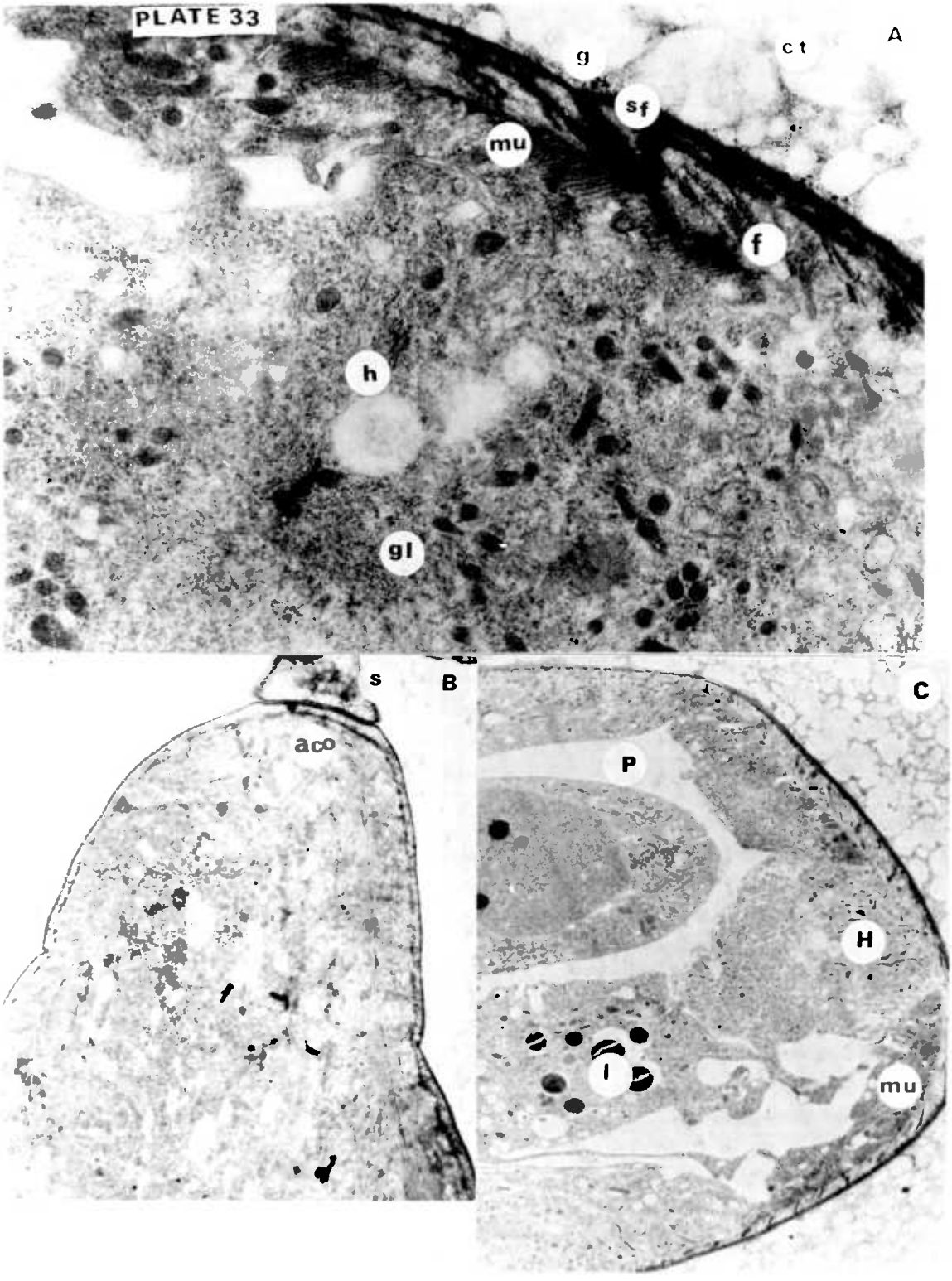


PLATE 33
A
g
ct
sf
mu
f
h
gl
B
s
aco
C
P
H
l
mu

PLATE 34

A. OBLIQUE LONGITUDINAL SECTION OF A DEVELOPING PARASITIC FEMALE *CONTORTYLENCHUS* FROM A PUPAL HOST

B. OBLIQUE LONGITUDINAL SECTION OF A MATURE ADULT PARASITIC FEMALE *CONTORTYLENCHUS* FROM AN ADULT HOST BEETLE

Key

bl	basal lamina
cf	column of fibres
ct	capsule tissue
db	dense bodies (lysosomes)
dg	dense granules
f	fibrillar layer
g	granular layer
l	lipid
M	membranous surface layer
mu	muscles
p	pseudocoelom
r	ribosomes
sf	fold of the surface layer
ve	vesiculate region

A. (X 25080) B. (X 20520)

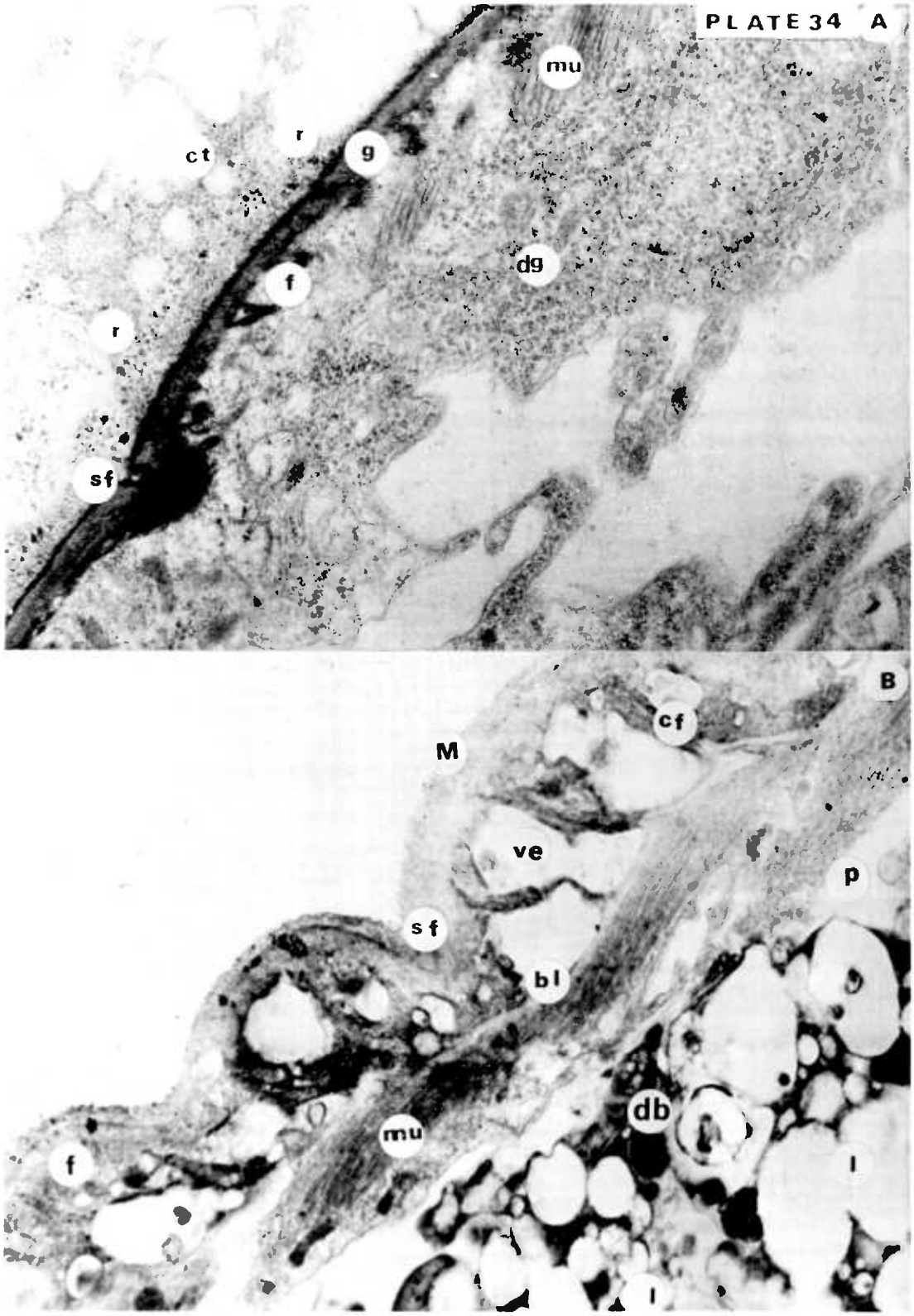


PLATE 34 A
mu
ct
r
g
dg
f
r
sf
B
cf
M
ve
p
sf
bl
db
mu
f
l
l

PLATE 35

TRANSVERSE SECTION THROUGH THE BODY WALL OF A MATURE PARASITIC FEMALE *CONTORTYLENCHUS*

Key

cf	column of fibres
d	dense bodies (probably lysosomes)
er	endoplasmic reticulum
f	fibrillar layer
gl	granular layer
h	hypodermis
l	lipid
M	membranous surface layer
mu	muscle
od	oviduct
w	whorls of membranes

(X 13680)

PLATE 35

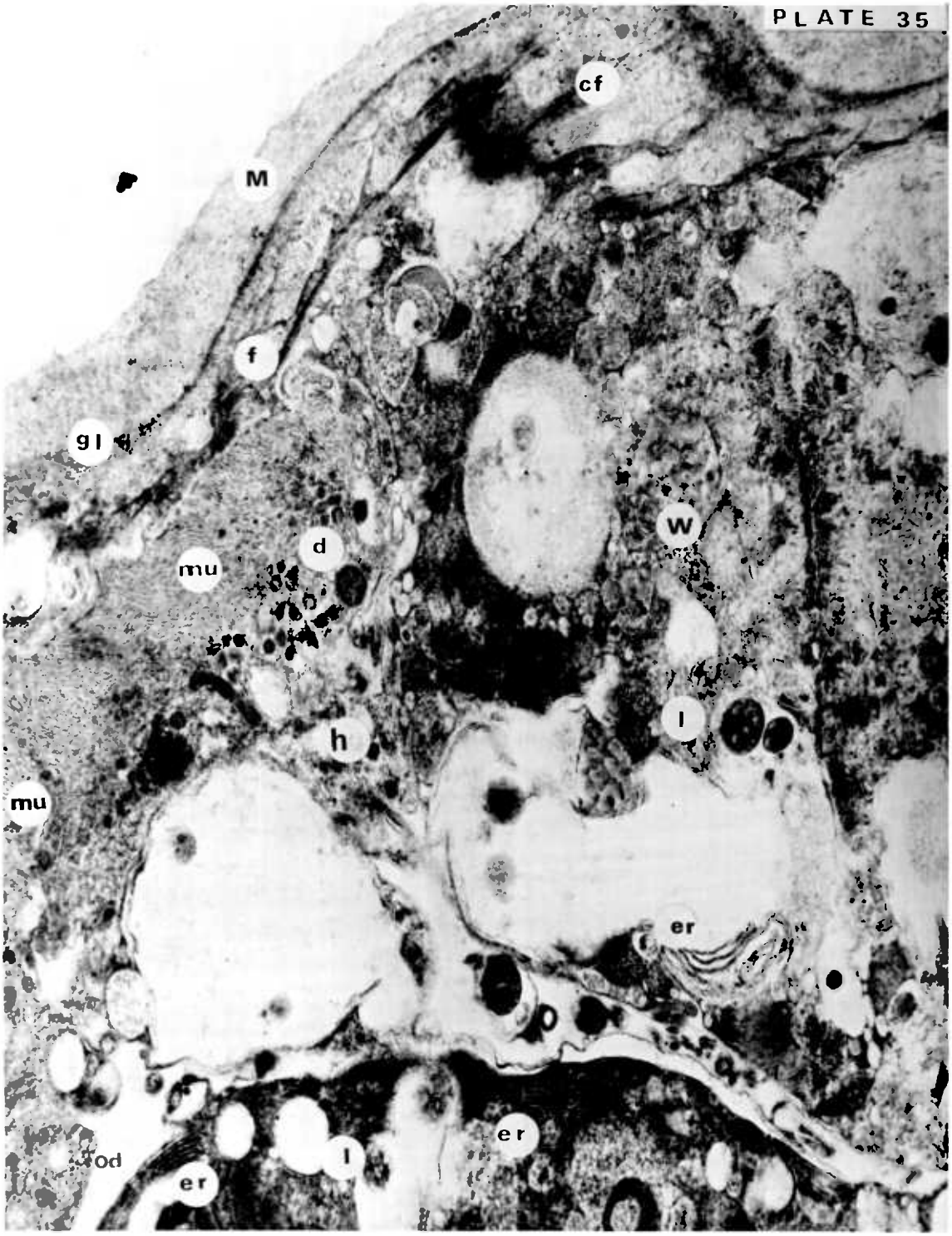


PLATE 36

TRANSVERSE SECTION THROUGH THE OESOPHAGEAL AND GLAND REGION OF A MATURE PARASITIC FEMALE CONTORTYLENCHUS

PLATE 37

TRANSVERSE SECTION THROUGH THE BODY WALL OF A MATURE PARASITIC CONTORTYLENCHUS

Key

ca	canaliculi
cf	column of fibres
f	fibrillar layer
g	gland tissue
gr	granular layer
h	hypodermis
M	membranous surface layer
l	lipid
oc	oesophageal cuticle
ol	oesophageal lumen
p	pseudocoelom
ve	vesiculate region
w	whorls of membranes

36 (X 24282), 37 (X 82080)

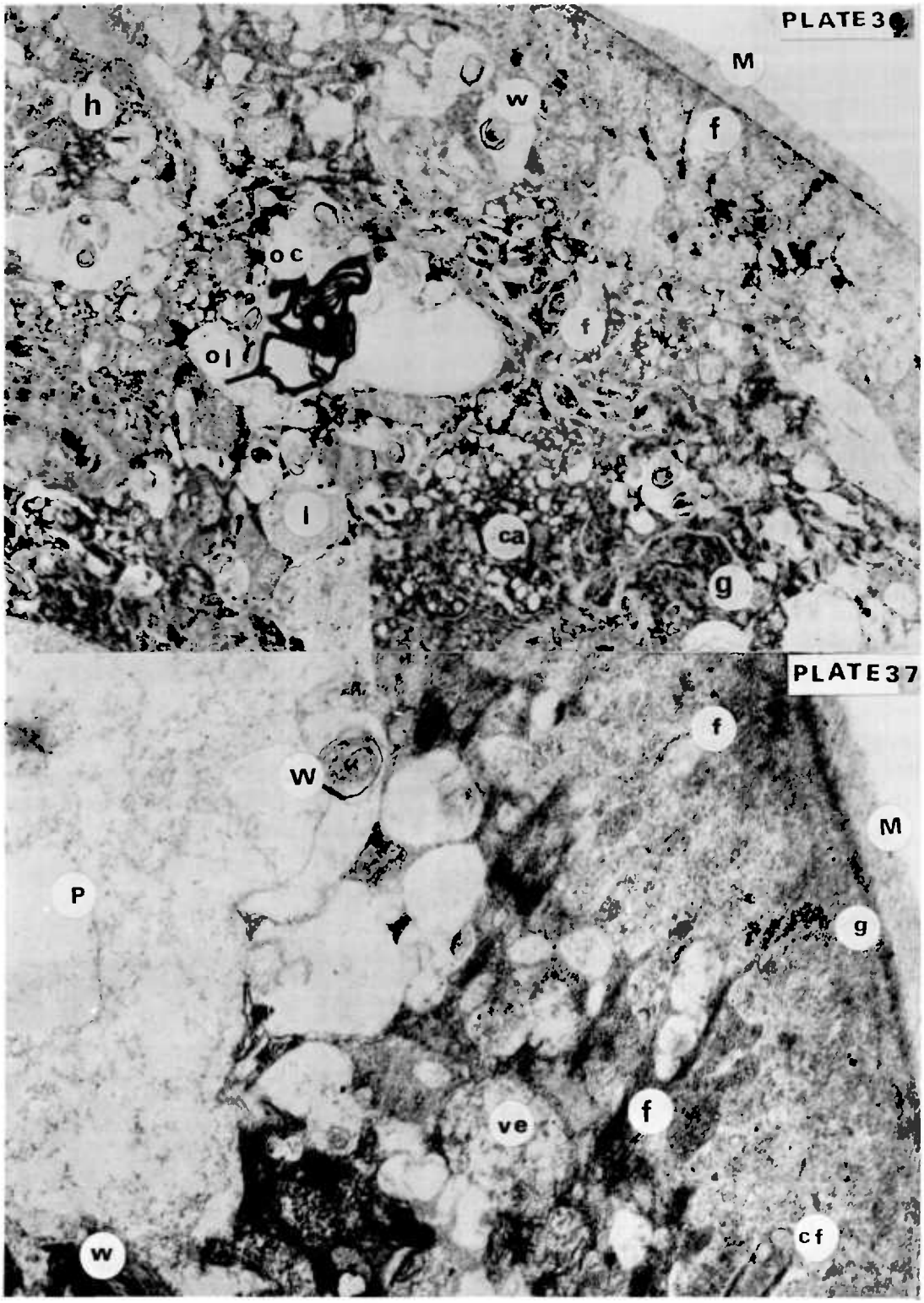


PLATE 36
h
w
M
f
oc
ol
f
l
ca
g
PLATE 37
f
W
M
P
g
f
ve
w
cf

PLATE 38

A. SECTIONS THROUGH THE BODY WALL OF A MATURE PARASITIC FEMALE *CONTORTYLENCHUS*

B. SAME AS A., BUT IN THE ANTERIOR REGION

C. OBLIQUE LONGITUDINAL SECTION THROUGH THE FOLD

D. OBLIQUE TRANSVERSE SECTION THROUGH THE FOLD

Key

bl	basal lamina
cf	column of fibres
f	fibrillar layer
g	granular layer
h	hypodermis
M	membranous surface layer
mg	mucopolysaccharide granules
mu	somatic muscles
p	pseudocoelom
ve	vesiculate region
w	whorls or membranes

A. (X 15960) B. (X 12141)

C. (X 12540) D. (X 13680)

PLATE 38

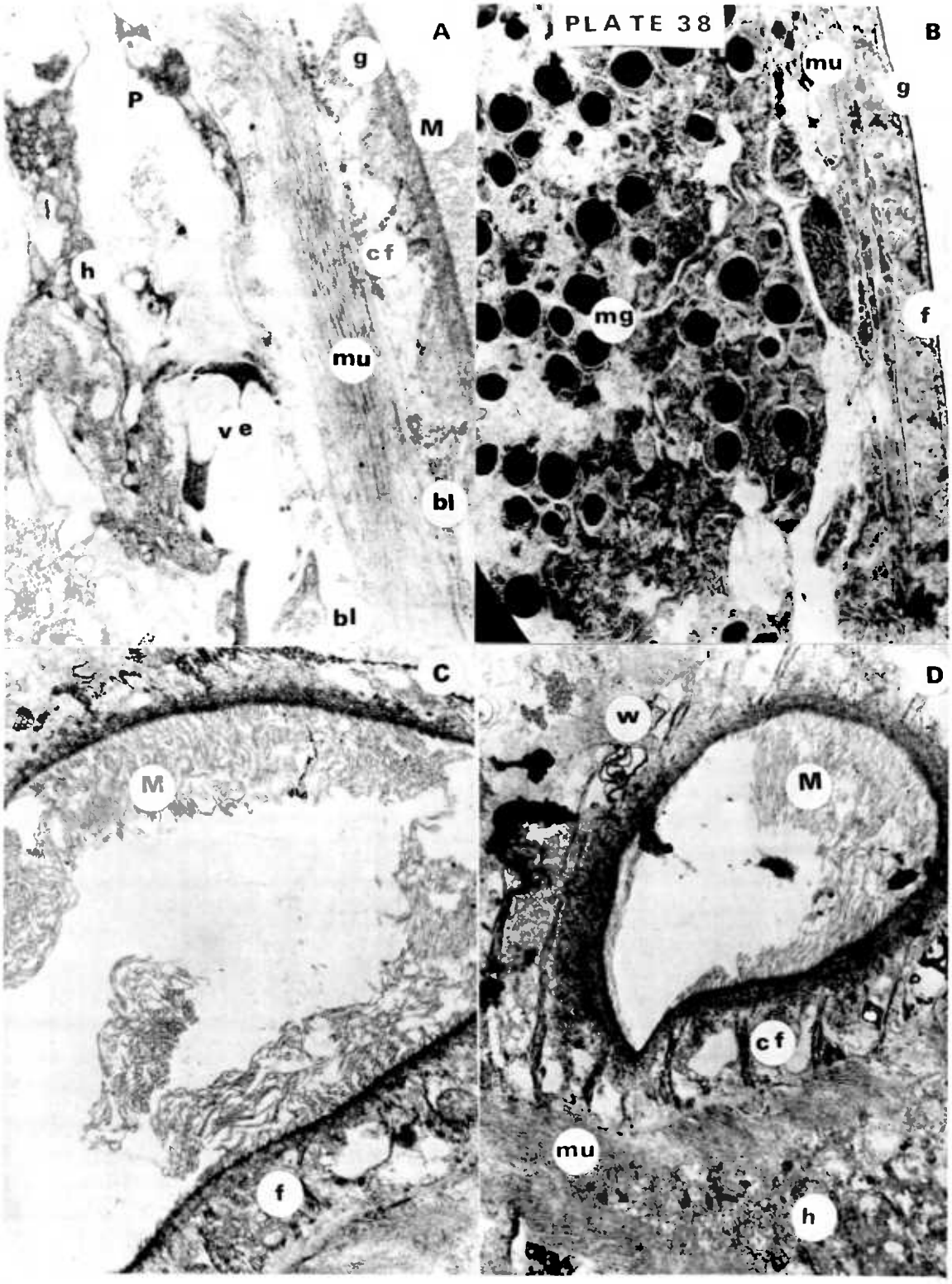


PLATE 39

A. TRANSVERSE SECTION THROUGH THE MATURE PARASITIC FEMALE *CONTORTYLENCHUS* IN THE FOLD REGION.

B. OBLIQUE LONGITUDINAL SECTION THROUGH AN OLD PARASITIC FEMALE *CONTORTYLENCHUS* IN THE FOLD REGION.

Key

f	fibrillar layer
sf	deep fold of the surface layer
l	lipid
w	whorls of membranes
mu	muscles
M	membranous surface layer

A (X 7600)

B (X 13680)

PLATE 39

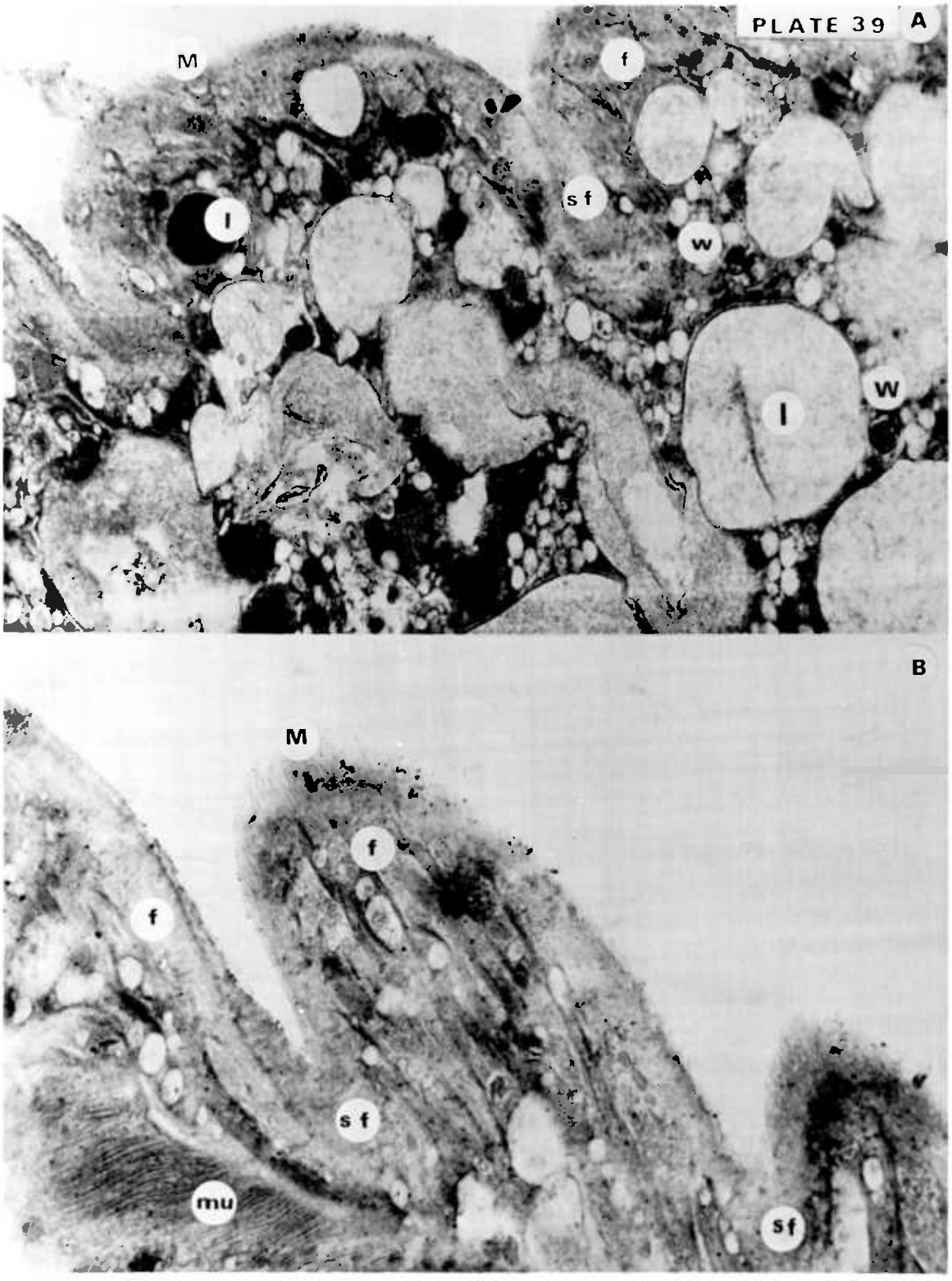


PLATE 40

1. LONGITUDINAL SECTION THROUGH THE BODY WALL OF A MATURE PARASITIC FEMALE *CONTORTYLENCHUS* IN THE FOLD REGION.

2. MAGNIFIED TIP OF THE FOLD.

3. MAGNIFIED MEMBRANOUS SURFACE COVERING.

Key

bl	basal lamina
cf	column of fibrous material
f	fibres
g	granular layer of the body wall
h	hypodermis
l	lipid
M	membranous surface covering
m	mitochondria
mu	muscle
sf	deep fold of the surface
va	vacuoles
w	whorls of membranes

Top (X 11172), Bottom left (X 6270)

Bottom right (X 85500)

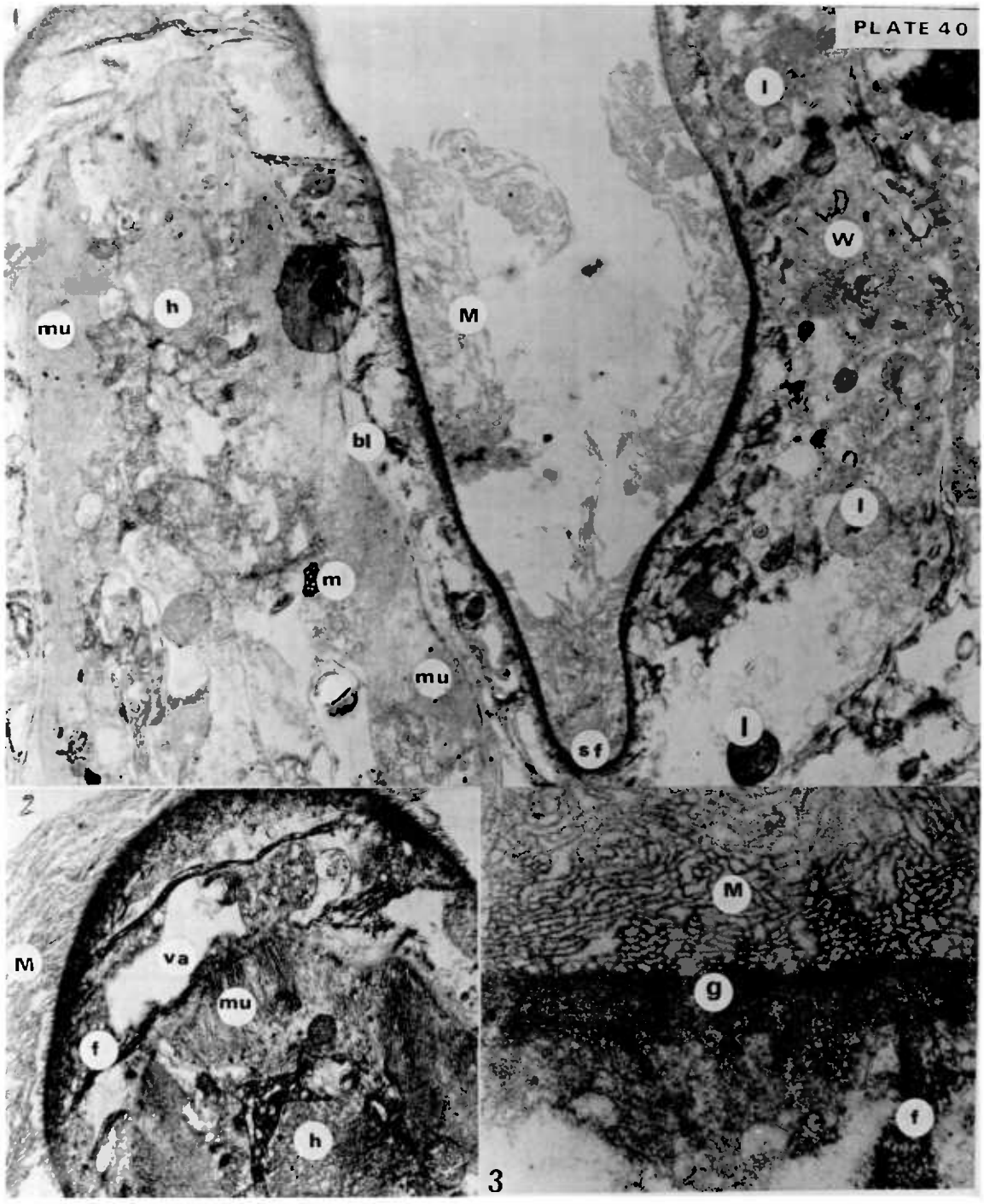


PLATE 40
1
l
W
h
mu
M
bl
l
m
mu
l
sf
2
M
va
mu
f
h
M
g
f
3

of platymyarian cells, each intercorded sector containing six cells. Glycogen mitochondria, and endoplasmic reticulum were found in this region.

The presence of mitochondria, glycogen and vesicles with dense contents which were membrane bound and rough endoplasmic reticulum (Plate 35) support the evidence that the outer region of the body wall is lacking in cuticle. The hypodermis at the anterior end contained four cords. The lateral cords were greatly enlarged and almost filled the body cavity (Plate 31 and 32). These hypodermal regions contained golgi bodies, lipid whorles or droplets, vesicles and mitochondria.

In cross sections of the developing parasitic female from host pupae the external surface of the nematode was covered by large and small dense granules (Plates 33A and 34A). These granules accummulated on the outer surface of the encapsulated nematodes within the host haemolymph, but these granules do not resemble debris. These electron dense bodies in later stages or after the growth of the parasitic adult female appeared like a membranous layer covering the whole external surface of the nematode including the fold region (Plates 33A, 34A B, and 40).

The membranous layer was present in almost all the mature parasitic females, but not in parasitic larvae or

the young developing adult from host pupa.

There is an indication that these dense bodies were released from the vesicles of the capsule tissue, then later they fused together forming this membranous layer, increasing the surface area of the nematode. It is also possible that the folds formed as a result of rapid growth of the nematode and also to increase the surface area for the same purposes (Plates 33A and 34A).

Contortylenchus diplogaster has no mouth, no anus and the oesophagus is a simple tube which is connected to the blind ending intestine. One dorsal and two subventral glands have been reported in the infective female. A transverse section in the oesophageal region from adult parasitic females from host pupae shows these glands and the oesophagus tissue (Plates 31 and 32). The oesophageal region contained many nuclei with dense patches of chromatin with nucleoli. The cytoplasm contained mitochondria and many small ducts lined with cuticle. An extensive canaliculi system together with numerous microtubules were found in this region (Plates 31 and 32). In cross section the oesophagus appeared as a circular tube lined with cuticle. There was no muscular tissue around or within the oesophagus which proves that it is a vestigal structure in adult parasites.

Groups of membrane bounded canaliculi were found in this area, scattered in different places around the oesophageal region (Plate 31). There are three main groups of canaliculi which belong to the gland tissue: these canaliculi are sometimes joined together forming small cuticularised ducts. The cuticle lining the oesophageal lumen appeared homogeneously electron dense (Plate 36). The oesophageal region is composed of 6 nuclei, some of them with nucleoli. There are also numerous mitochondria vesicles and vacuoles, dense granular minute bodies and the canaliculi of glands. Unfortunately, sections were not obtained to show the junctions between these glands and the oesophageal lumen. The cytoplasm of the oesophageal region was packed with small and big electron dense granules (Plates 31, 38 B), varying in size from 0.45 to 0.89 µm in diameter. From the histochemical study it seems more likely that these granules are mucopolysaccharide, fine glycogen particles were also seen around these granules (Plates 31 and 38 B). Gland tissue, in Plate 36, Plate 31 showed the oesophageal region and the canaliculi of the glands. Plate 31 and 32 showed the small cuticularised ducts and the lumen.

PLATE 41

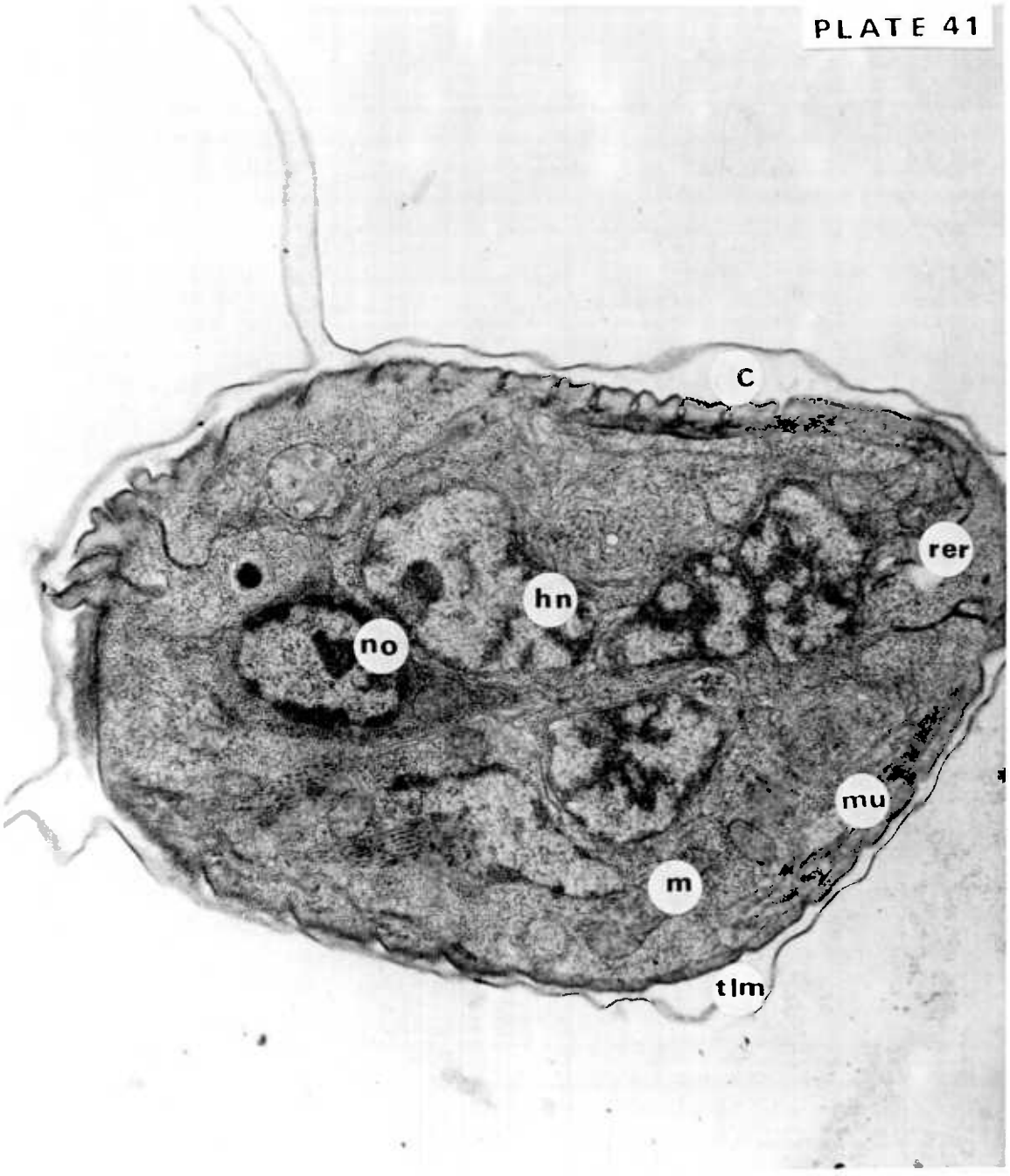
A TRANSVERSE SECTION THROUGH *CONTORTYLENCHUS* PARASITIC LARVA IN THE LATERAL FIELD

Key

C	cuticle
hn	hypodermal nucleus
m	mitochondria
mu	muscle
no	nucleolus
rer	rough endoplasmic reticulum
tlm	triple layer membrane

(X 20520)

PLATE 41



4.6. DISCUSSION

Ips sexdentatus inoculated into pine logs, bred better in ventilated logs. The small culture chambers employed by Al-Rabiai (1970) were inadequate because only a few beetles were found to reproduce and these usually died within a few weeks. The beetles were very small and very weak, and they were unable to react against the nematode infection. It is likely that these beetles were not strong enough or healthy to overcome the invasion. At 26°C with a relative humidity of 70-75%, the modified log method provided a satisfactory source of material for experimental work. The beetles emerged within a month even when they were infected by Contortylenchus.

Al-Rabiai (1970) was the first to record Contortylenchus sp. in the haemolymph of I. sexdentatus. The nematode becomes encapsulated during the first week after the beetle's emergence, although she thought that the infective adult female Contortylenchus penetrated host pupae. Few observations were obtained of the encapsulation of nematodes in the pupal host, from forest populations, and laboratory stock. Al-Rabiai (1970) believed encapsulation occurred only in adult beetles. This investigation, however, has shown that

the pupal host does react against the invading foreign bodies as soon as invasion takes place. There would be no value if this immune reaction took place after the parasites have been in the host's haemolymph for more than one week, since haemocytes are usually immediately stimulated and mobilised.

-- The relationship between C. diplogaster and its host I. sexdentatus would, according to Crofton's (1971), definition for parasitism, represent an equilibrium between the host and the parasite, based on evolutionary adaptation, which ensures the continuity of their relationship by the survival of an unharmed host. As an adaptation to parasitism in nematodes after invading their host, part of the system controlling development may have been lost and the parasites may be dependent upon host regulation. Rogers (1963) gave evidence which supports this hypothesis, when he said that development stops when the infective stage is reached, it is then resumed in the next host. That means the host may provide a stimulus which acts on the post-infective female to produce something necessary for the resumption of the development as a parasitic stage. Since C. diplogaster develops and grows rapidly after being in the host haemocoel, this mechanism is likely to occur.

In this study, a survey has been made of the forest population of Ips sexdentatus in two localities, and it was found that the incidence of parasitism and encapsulation varied considerably according to the seasonal fluctuations in these two places. These variations were probably attributed to the environmental conditions, such as moisture, temperature and the nature of the pine bark. The rate of parasitism was found in both sexes to be comparable. The male hosts tended to have the highest numbers of parasites. Al-Rabiai (1970) stated that equal chances of infection with Contortylenchus for both sexes were obtained, although she found that the females tended to have the higher number of parasites. This study found the reverse: the male beetles were found to have more parasites than the females and the highest records of multiple infections were from male beetles, whether from forest populations or in the laboratory stocks. As many as 60 and 150 adult parasitic female nematodes were recorded from males from the forest and laboratory populations, respectively. While the highest number of parasites recorded from the female beetles was 45 females in laboratory stocks. It is possible that the males were able to withstand higher parasitic burden than the females, but no further explanation can be given for the increased infections of the male beetles. These experi-

ments proved that there was no significant differences between the rate of parasitism by Contortylenchus of male and female hosts, confirming Al-Rabiai's (1970) findings.

Up to 83 adult Contortylenchus spp. have been found in a single adult beetle (Ruhm 1956). This is the highest number of nematodes so far recorded from the body cavity of a bark beetle. Riding (1971) reported multiple infections in phorid flies Megaselia halterata, of 28 female Howardula sp. in one insect. Sixty-five adult parasitic females of the same nematode were recovered from one host fly M. halterata. The size of these parasites was greatly reduced when there were multiple infections and they were unable to reproduce (personal observations, 1971). Welch (1956) pointed out that the rate of infection in bark beetles varies considerably and is dependent on the host density and the activity of the pre-infective parasite. Infection of Ips by Contortylenchus took place during pupation, but why the infective female nematode was not found in larval beetles which occupied the same galleries is not understood. Not a single Contortylenchus was ever found in the haemocoel of the beetle instars. On the other hand, Contortylenchus found in the pupae were encapsulated in

the haemolymph, often several post-infective nematodes were found in a single capsule (Attraction between the parasite and its host may occur; it is likely that the nematode is attracted to the pupa to gain entry). There is probably a synchrony between the development of the infective nematode and the pupation of the beetle (Al-Rabiai 1970) (The relationship between the parasite and the host is highly specialised since in I. sexdentatus the parasite was absent from beetle instar stages but present in the infected beetle pupae).

The incidence of parasitism of the beetle is dependent upon several factors, but the most important and most obvious is the level of infection in the parents. The likelihood of infection is related to the distribution of the pupae in the brood galleries and the distribution of the pre-infective nematodes in the tunnels. The female beetle galleries crossed the larval galleries permitting the transmission of the nematodes from adult beetles to beetle pupae. Infected females Ips tended to have short galleries compared to uninfected female beetles. It seems that the broods were reduced in size because of the infection which resulted in a higher intensity of parasite in each individual host. Similar observations were obtained by Al-Rabiai (1970) and Finney (1970). In

I. confusus the brood was reduced to about one-third of the normal number and the gallery was shorter when they were infected by C. elongatus (Nickle 1963). It was observed in heavily infected females of S. ventralis by S. elongatus resulting in aberrant gallery construction. Short egg galleries were formed by moderately infected females but lightly infected females constructed longer galleries (Ashraf and Berryman 1970).

Nematode parasites associated with insects are affected by environmental factors, host density and the activity of infective female nematodes, which subsequently effects their rate of infection. Postnikova (1960) reported higher parasitism in moist localities and the infection rate was also affected by seasonal fluctuation. He stated that parasitism of fleas by Heterotylenchus pavlovski increased from a minimum in the spring to the maximum in October. Incidence of parasitism in M. halterata by Howardula was also effected by seasonal fluctuations (Hussey, 1964; Riding, 1971; and personal observations).

Environmental conditions within the log will not fluctuate as widely as those on the immediate surface. The number of infected beetles by C. diplogaster obtained in the laboratory varied considerably, since the parasitism

of the beetle introduced to the logs was not determined. A high incidence of parasitism was obtained in laboratory populations mainly because the pieces of logs used were not longer than 1.5 ft. long, introduction of 4 to 8 male and female beetles of which about 50% were infected. Because of the effect of parasitism on the host population infected and uninfected beetles were used to keep the population going. As many as 150 adult females were obtained from a single host. One can infer that when few progeny were obtained and the majority of these were infected then presumably the adults originally inoculated were all infected and vice versa.

Effects of parasitism on the host

The damage inflicted to insects by their parasites are commonly concerned not only with their external and internal morphology but also with their physiology, development, and behaviour (Wulker 1964). Parasitism in Ips sexdentatus by C. diplogaster resulted in the inhibition of normal development, reducing fecundity and restraining the activity of the beetles. I have been unable to detect any effect on the external features of the beetle other than the colour of the adults due to the presence of C. diplogaster. Infected adult beetles were lighter in colour than normal beetles of the same age, suggesting that parasitism retarded the development of

the host.

The effect of parasitic nematodes on their respective insect hosts has been studied by many investigators. Wulker (1923) never observed any decrease in size of parasitised beetles Hylobius abietis by Allantonema mirabile compared with the normal host: they always had well stocked reproductive organs with ripe sex-cells. Small and large specimens of both sexes of I. sexdentatus harbour the parasite Contortylenchus. Welch (1956) was unable to detect any external effect caused by the parasites, no morphological differences appeared on the body. Swelling of the abdomen was noticed, sometimes in the infected flies, but the same feature appeared in the gravid female flies. Goodey (1931) was unable to detect any effect on the external characters of the host due to the presence of Tylenchinema oscinellae. Parasitized Megaselia halterata by Howardula sp. had swollen abdomens and transparent cuticles, (Personal observations and Riding, 1971). The transparency of the cuticle occurred as a result of the diminution of the fat bodies, which enable the detection of the parasites inside the host. In I. sexdentatus dissection was the only way for detecting the presence of the parasites and the differences in the colour of the adult beetle from the same age.

Emergence of I. sexdentatus was delayed by nematode infection. The beetles that emerged earlier were either non-parasitised or had a low percentage parasitism, while those which emerged later had a higher percentage of parasitism. The hypothesis that nematodes delayed beetle emergence could explain these observations. Similar observations were obtained in I. sexdentatus by Al-Rabiai (1970) Finney, (1970) and in Deudroctonus brevicomis, bark beetles parasitized by C. brevicomi (Massey) (Nickle 1963). Ashraf and Berryman (1970) stated that S. elongatus caused delayed emergence, limited flight and aberrant attack behaviour of infected Scolytus ventralis. Welch and Bronskill (1962) found that emergence of mosquitoes was retarded by nematode infections.

More convincing evidence as to the effect of parasitism was seen internally. In almost all cases, irrespective of parasitic burden, the fat body of the beetle was diminished in size and in heavy infestations it was non-existent. Salt (1963) pointed out that parasitism had a considerable effect on the condition of the fat bodies which were required for flight, reproduction and development.

One would assume that several adult females of C. diplogaster with their progeny in the body cavity of

single host beetle would cause a definite drain on the reserve food of the host and probably additional damage because of their waste products. This assumption was supported by serial transverse sections which showed that sections of parasitized beetles had fewer fat bodies compared with unparasitized ones. This is clearly visible in dissected beetles. Parasitism caused severe depletion of the host fat body stores (Al-Rabiai 1970; Riding 1971; Hudson 1972). In adult desert locusts, Schistocerca gregaria infected with nematode Mermis nigrescens a significant reduction in the level of glycogen and non-glycogen carbohydrate in the fat body and the total carbohydrate in the haemolymph was severely depleted during the active growth of the nematode (Gordon, et al 1971; Gordon and Webster 1971). In a preliminary study using gel electrophorsis the effect of parasitism on the haemolymph proteins was demonstrated. New protein bands appeared in infected beetle haemolymph compared with the control, but enough observations were not obtained because of the shortage of time. It seems of interest to note that the effect of parasitism by C. diplogaster on I. sexdentatus haemolymph proteins. Since the results were different as the parasites density increased, where new thick bands appeared.

I. sexdentatus infected were less able to develop or reproduce. Multiple infection always resulted in an inability of the beetles to leave their logs and overlapping populations always occurred within the same log. Heavily parasitized beetle had reduced fecundity of the female. The greatest damage occurs to the females when the progeny start leaving the host via the alimentary duct when they damage the thin peritoneal sheath and destroy it by their activity. As a result the ovarioles appear to hang loose in the body cavity. Highly parasitized host females have less developed ovaries and they are probably unable to reproduce, suggesting that the development of the reproductive organs may be effected directly by nutrient starvation or indirectly through the endocrine system (Thomson 1952). The retarded development of parasitized beetles probably resulted from the reduction in the protein concentration in the haemolymph.

Disintegration of cells around embedded nematodes suggested that feeding was taking place by the secretion of histolytic enzymes (Lee 1965).

Fuchs (1915) and then Massey (1960) studying Tylenchus contortus (C. diplogaster) parasitizing I. typographus found a reduced egg output in the female was about half the normal. Fully developed ovaries were

observed in non-infected adult females of the face fly Musca autumnalis while females of the same age infected with Heterotylenchus autumnalis had not developed (Stoffolano 1970). No eggs were laid by Scolytus ventralis parasitized by Sulphuretylenchus elongatus and there was a drastic reduction in egg production by Deudrocto nus obesus parasitized by nematodes (Massey, 1964, 1969). C. elongatus reduced the egg production of I. confusus (Massey 1960; Nickle 1963; Ashraf and Berryman 1970). Hudson (1972) found parasitized adult sciarids Tetradonema plicans had no gonads.

The phenomenon of host intersex was not observed in infected I. sexdentatus, but this phenomenon was observed by Wulker (1961) and it was accredited to mermithid parasitism.

Effect of parasitism on the parasite

The fact that the degree of infection influences the fecundity of the females has already been mentioned. The same is true for the development of the parasite (Taliaferro, 1940).

As the infection of I. sexdentatus with C. diplogaster becomes heavier, the survival, the rate of development, and the final size of the worms decrease. The volume of the parasitic female of Contortylenchus was related to its

own burden in the host. Solitary parasitic adult females were bigger than individuals recovered from a host with many parasites; presumably there is an intraspecific competition between individuals, for the amount of food or space available in the host. The development of the parasitic nematode or its progeny is intimately related to the development of its host and influenced greatly by the physiological and nutritional state of the host. When the environment of the host is not suitable it will affect its physiology which in turn influences the parasites. Starved beetles withstood stress for a month when they were uninfected. While parasitized beetles were found to be affected by the density of parasitism. Mortality time was found to be related to the parasitic burden per host. The parasite died soon after the death of its host. Heavily parasitized beetles were the first to die due to insufficient food, and a deteriorating condition. Van der Bosch (1964) showed that starvation had a physiological effect on the host, when he found that fully fed larvae of Hypera brunneipennis clearly showed greater encapsulating capacity than those which were starved.

Superparasitism or multiple infection are widely known to effect the size and the fecundity of parasites

(Cross, 1966) as was observed in C. diplogaster (in the result section). Welch (1963) pointed out that as the mass of the parasites is closer to that of the host in invertebrates than it is in vertebrates, so the "crowding effect" is much clearer in the first group.

The total progeny increased with each extra parasite but the individual parasites produced fewer progeny. This study proved that when 60 and 150 adult parasitic Contortylenchus were found in one host, these parasites were unable to reproduce and they were smaller in size. Limitation of the parasites was probably controlled by competition for nutrients and oxygen.

Host reaction

A great variety of metazoan parasites live temporarily in the haemocoele of insects. These include larvae of trematodes, acanthocephala and most of the super families of nematodes. Some of them appear to use mechanisms of resistance similar to those used by parasitoids (Salt, 1968).

The encapsulation of insect parasites has been described by workers, dealing with the incidence of reactions in naturally parasitized host, the cytology of the cyst and others (Lartschenka, 1933; Boese, 1936; Schneider, 1950) suggested that capsules fall into two

groups, one has thick colourless jelly-like walls of which the cells retain their nuclei. The other has thin, tough, brown walls formed of dead tissues.

I. sexdentatus made an uneffective defence reaction to C. diplogaster infection. The fact that the parasite in the haemolymph within the capsule developed and reproduced, suggested that this reaction did not appear to be antagonistic to the nematode. Similar observations have been found by Welch (1956) in Drosophila phalerata infected with Resonema coronymphum, and similar to the reactions of insect hosts to habitual parasites (Salt 1963).

Sufficient observations have been made in this study to permit some generalizations on the nature of encapsulation and to review the importance of this reaction as a defence mechanism. Various types of host blood cells contributed to the encapsulation response, including: plasmatocytes, amoebocytes and granular haemocytes. It was found that these blood cells become stretched around the parasite within the first 24 hours (1 day old pupae have completely encapsulated parasites). One hundred percent encapsulation occurred in host pupae irrespective of the density of the parasite per individual host. Al-Rabiai (1970) stated that encapsulation

occurred only after the emergence of the adult beetle but I found it in pupae. Fuchs (1915) described Tylenchus contortus (= C. diplogaster) in the adult beetle I. typographus; in which the parasite was surrounded by a cellular capsule containing fine tracheoles, and covered by larger tracheae. Fuchs indicated that the parasite continued to deposit viable eggs and that hatched juveniles later bored through the capsules.

One of the factors that affect the occurrence of encapsulation of parasites in their host is superparasitism (Salt 1963; Kitamo 1969). Percentage encapsulation declined in the adult host, although it has been observed that the number of empty capsules which were found in the haemolymph of the adult beetle corresponded with the number of free adult parasitic females. This indicated that 100 percent encapsulation had occurred at the beginning of the reaction. Excessive parasitism or a limitation in the number of host haemocytes may have caused thin capsules in which the activity of the larvae caused emergence. THCs from pupal host were greater than in adults which explain why the encapsulation declined in the adult host where it was found that the likelihood of a parasite being encapsulated was inversely

related to the degree of infestation. The host's powers of encapsulation may be limited in the case of multiple infections. Salt (1959) stated that as the number of parasites increase the haemocytic reaction appears to decrease in intensity. Physical resistance to encapsulation by vigorous movement may explain the freedom of active nematodes from encapsulation (Salt 1963).

Drosophila melanogaster larvae invariably encapsulate all the eggs of the parasite Pseudeucoila mellipes even when heavily parasitized (Stream and Greenberg, 1969). As many as seven encapsulated P. mellipes have been found in a single pupa of D. melanogaster. The failure of superparasitized adult hosts to encapsulate all C. diplogaster may be due to their being weakened by the excessive parasitism. Weakening of the adult host must be caused by the demands made by the parasites during their development and reproduction. Superparasitization can be advantageous, leading to more surviving progeny than solitary parasitization. Superparasitized hosts containing only susceptible parasite eggs, fail to encapsulate some of them (Streams 1971).

In host-parasite associations encapsulation occurs in both solitary and superparasitized hosts, generally

the frequency of encapsulation is much lower in super-parasitized than in solitarily parasitized individuals (Puttler and Van der Bosch, 1959; Puttler, 1967; Streams 1968).

Encapsulation of S. elongatus eggs and first stage larvae by S. ventralis was observed only in the body cavity of the 1st and 3rd instars. These capsules were enclosing individual groups of nematodes and they appeared to be surrounded by blood cells (Ashraf and Berryman, 1970). As many as 36 capsules were found in one adult I. sexdentatus. Forty-five capsules were found in one larva of S. ventralis, (Ashraf and Berryman 1970). They also stated that multiple encapsulation appeared to be more common than individual encapsulation. Up to 8 adult C. diplogaster were found in one capsule. Poinar (1965) found 15 - 20 nematodes in a single capsule in the body of sciarid fly.

The histochemical investigations of the capsule wall showed that the major component of the capsule wall was protein which was mainly derived from haemocyte structures. The positive reactions for Best's carmine and PAS indicated the presence of glycogen or mucopoly-saccharide. The negative results obtained for lipid, suggested that the capsule is not formed by fat bodies of

connective tissues of the host. Bruce (1970) concluded from the histochemical study of the capsule of Trichinella spiralis in host muscle that it consisted mainly of collagen and cystine. Bruce suggested that the basic framework of the capsule wall is the sarcolemma and basement lammella of parasitized muscle fibre. Poinar et al. (1968), demonstrated acid phosphatase activity localized in cell membranes and lysosome in the capsule around a mermithid formed in Diabrotica (coleoptera). Poinar also suggested that the pigmented material surrounding the parasites was melanin.

Cytoplasmic mucopolysaccharide has been reported in the insect's haemocytes, Rizki (1957) noted the presence of mucopolysaccharide in the plasmatocytes of the fly D. melanogaster. Neutral mucopolysaccharide was found in the basophilic granulocytes of the beetle Allomyrina dichotomus and in the amoebocytes of the hemipteran Rhodinius prolixus (Wigglesworth 1959). The histological studies of the capsules in I. sexdentatus showed that the capsule develops gradually to a non-cellular envelope containing mucopolysaccharide. The amoebocytes were also found in the haemolymph of I. sexdentatus; it is possible that these haemocytes secrete this compound in the capsule since they have been reported to be involved in

the formation of capsules.

In cases where a haemocytic response to parasitization occurs, deposition of melanin or melanin-like material around the parasite may occur (Thorpe, 1936; Bess, 1939; Muldrew, 1953; Salt, 1955, 1956, 1957, 1963, 1970; Salt and Van der Bosch, 1967). Eckstein (1931) was the first to study melanin formation in a parasitized insect. He concluded that insect blood contains an inhibitor of tyrosinase in its haemolymph.

Melanin formation in I. sexdentatus may be inhibited by the parasite itself secreting the mucopolysaccharide that has been reported in its anterior region in the early stages of capsule formation. Salt (1956) stated that there may be encapsulation when there is no melanization or when melanization is blocked, but melanization does not occur without the haemocytic response. In fact, Salt (1963) considered the "cellular reaction as a defence reaction proper" and looked upon the "deposition of melanin as a secondary development".

The involvement of mucopolysaccharide in the cellular response to invasion led Salt to comment that, "the connection of polysaccharides and particularly mucopolysaccharide with both insects and mammalian cellular defence reaction seems too frequent to be more than

coincidence".

The mechanisms of melanization are very complex and both organic and inorganic inhibitors have been recorded (Rendell, 1972). Mechanisms by which the melanization process could offer protection against invading organisms have been described. Formation of melanin itself or products of the reaction seem to be responsible for killing encapsulated parasites (Salt, 1970). Melanin formation is initiated by tyrosinase acting on tyrosine. Quinones are produced as an intermediate compound in the melanization process, notably dopaquinone, which could act as a generalised antibodies (Taylor, 1969). An oxygen carrier within the haemolymph could induce the oxidation of tyrosine to 'dopa' or through to dopaquinone, the former by acting as hydrogen acceptor (De Carvalho Lima, 1965).

It can be postulated that melanization in I. sex-dentatus has been inhibited by mucopolysaccharide, whether released by the haemocytes or secreted by the parasite. This suggestion is supported by observations obtained by Rendell (1972) who suggested that mucopoly-saccharide may be involved in the inhibition of melan-ization. He also stated that melanization inhibitor has characteristics similar to those of the bactericides of insects.

The role of mucopolysaccharides in the cellular defence mechanisms of encapsulation has been considered in detail by Salt (1970). His conclusions indicated that any material introduced into the haemocoel which has an effect on the surface of the haemocytes could stimulate the release of mucopolysaccharides, this includes foreign particles of material as well as chemical substances. Salt (1970) observed that melanization in capsules only occurred after cell breakdown. This supports the findings in this study where the lysis of cells took place and the cells (haemocytes) which contain all the factors for melanin production were reported to be present in the haemolymph of I. sexdentatus but its formation was inhibited probably by mucopolysaccharide. Poinar et al (1968) regarded melanin formation as an integral part of the immune response. Poinar and Leutenegger, (1971), stated that melanin could occur on the parasite before cells were attached to it - so-called "humoral melanization".

In larvae of Musca domestica parasitized by the nematode Heterotylenchus autumnalis, large numbers of haemocytes circulate throughout the haemocoele and encapsulate the parasites (Nappi and Stoffolano, 1971). The initial reaction is the aggregation and fusion of

oenocytoids to form a pigmented layer which adheres to the cuticle of the parasite (Nappi and Stoffolano, 1972). Nappi (1972) found that nematodes were melanized and in various stages of encapsulation in all host larvae Orthellia caesar.ion.3days after infection.

A high aminoacidemia is a characteristic of the insects (Florkin and Jeuniaux, 1964). The increasing importance of free amino acids as haemolymph constituents appears as an evolutionary tendency developed in the most evolved groups such as Lepidoptera, Hymenoptera, and Coleoptera. In these insects contrary to that obtained in vertebrates and other invertebrates the composition of the internal medium is thus similar to that of the cells (haemocytes) (Florkin and Jeuniaux, 1964).

This study has shown that the parasitic female C. diplogaster and her progeny grow rapidly within the capsule in the host haemolymph. The nutritional demands were made upon the host by the developing parasite. The amino acids are the most important source of protein for the nematode in this ambient. Eighteen amino acids were found to be present at different concentrations. Glutamic acid was most common, whilst butyric acid was scarcist. All the amino acids in the haemolymph of some insects (Florkin and Jeuniaux, 1964) appeared in the capsule except

tyrosine, butidine and butaric acid, suggesting that the capsules are definitely formed by the haemocytes of the host; their composition was said to be similar to that of the haemolymph (Florkin and Jeuniaux, 1964). The amino acids are present in different concentrations according to the species. Concentration of tyrosine in the haemolymph varies widely during the whole life of insect. These variations are related to the utilization of tyrosine in the protein-tanning and melanization of the new cuticle (Florkin and Jeuniaux, 1964). These reasons may explain the absence of tyrosine in the capsule.

Insects have given up the physiological association between respiratory and circulatory systems. The tracheal system ensures the arrival of oxygen in the immediate vicinity of the cell, so their haemolymph is not concerned with oxygen transport nor with the transport of CO_2.

C. diplogaster caused a drastic effect on the oxygen consumption of its host. Heavily parasitized hosts consumed more oxygen than uninfected or lightly parasitized hosts which suggests that the extra oxygen is consumed by the parasites present in their haemolymph. Infective stages of Strongyloides papillosus which enter the host

by routes where oxygen is freely available are so susceptible to lack of oxygen that they have been called strict aerobes (Rogers and Sommerville, 1963). It is likely that C. diplogaster are strict aerobes, since it was shown they consumed oxygen and died when an inhibitor was used. However, many species which enter the host via the gut where the pO_2 is low, as in Ascaris lumbricoids, can survive the absence of oxygen for some weeks (Rogers and Sommerville, 1963).

Complete cellular encapsulation has no permanent effect upon some parasites. The survival of these parasites may be partly due to their inactivity and to the oxygen requirements in which the capsule does not prevent a sufficient transfer of gases (Salt 1963). In the capsules surrounding C. diplogaster, large tracheae and fine tracheoles have been found covering and ramifying the capsule from which the parasites were supplied with oxygen. Doutt (1963) stated that in tachinids a funnel-shaped structure was formed after the parasite penetrated the host at the site of penetration and he considered it as a respiratory funnel.

Fuchs (1915) found that fine tracheoles participate with the matrix which grows around the parasites making a fine net around them. It seems probable that death of

encapsulated parasites results from suffocation. Branches of tracheae have fequently been observed to have grown towards capsules (Askew 1971). Growing of tracheoles on the capsules indicate that there is an oxygen shortage in their vicinity.

It was found in this study that capsules sometimes remain free in the haemocoele, and fall out when the host is dissected in entomological saline. They may, however, be fastened to various organs by connections made with blood cells to fat bodies, malpighian tubules and tracheae. On the other hand capsules often have tracheoles and trachea included in their substances especially on the surface and within the matrix. These observations are confirmed by the results obtained by many authors. Marchal (1904) worked with caterpillars of Hyponomenta, Fuchs (1915) and Vulker (1923) in the beetles Ips and Hylobius, Seurat (1916) in Periplaneta orientalis. Thorpe (1936) claimed that haemocytes are important in forming such tracheol or respiratory structures when he studied the respiratory interrelation between an insect parasite and its host (Chalcid and Coccidae). Thorpe stated that the tracheal attachments are of no value to the parasite and it is not an adaptation. The development of tracheal attachments in the capsule of C. diplograster

could be stimulated by the parasites as their presence in large numbers evoked a sudden activation to the tracheal epithelium of the host. The parasites may secrete substances which stimulate the tracheal epithelium of certain sites, or changes in the respiratory activity may occur, and reduction in oxygen tension, because of the parasite.

It has been shown in this study that the cellular capsules in I. sexdentatus haemolymph surrounding the parasite C. diplogaster present different aspects at different stages of their development. The complete cellular capsule from young adult beetle was composed of three layers. The cells taking part in the process of encapsulation have been described by almost all workers to be blood cells although different names have been used (Durham, 1891; Holland, 1920; Cameron, 1934; Thompson, 1915; Boese, 1936; Salt, 1963, 1970; Grimstone et al, 1967; Nappi and Stoffolano, 1971; Nappi, 1970; Stoffolano and Streams, 1971).

The electron microscopic study of the capsule showed that it forms the haemocytes flattening themselves against the parasite. A fully formed capsule appears lamellate owing to the flattening of the haemocytes.

The subject of encapsulation is complex, involving

many considerations. Certain generalisations have been made by Salt (1963). In general, hymenopterous parasites are not encapsulated in their normal hosts, and do elicit defence reactions in unusual hosts. This generalisation cannot be extended to C. diplogaster, for the parasitic female and their progeny live in their usual host in a respiratory sheath, which is probably a modification of the host's defensive reaction. C. diplogaster successfully meet the defence reactions of their hosts by diverting them. However, it seems that I. sexdentatus possess a defensive reaction which does not require conditioning to this parasite, and the parasite overcomes this reaction in its usual host. There must be strong opposing, selective pressures operating; pressure to overcome the parasite on the part of the host and pressure to evade the defensive mechanism on the part of the parasite (Askew, 1971).

A successful parasite like C. diplogaster must circumvent the host's defensive reaction in some way. Mercer and Nicholas (1967) stated that encapsulation does not prevent the growth and development of Moniliformis dubius which must feed through the capsule. Similar results have been demonstrated in this study. The parasite may be unable to survive without the capsule at

the beginning, this capsule is able to osmoregulate in the host's haemolymph. It has been suggested by Salt (1957) that osmotic differences between the capsule and the host's haemolymph create an exosmosis causing the death of the parasites Nemeritis in the wasp Callipora. Muldrew (1953) suggested that the capsule exert a detrimental effect on the parasite embryo and inhibit its development due to the permeability phenomenon.

The initial reaction in I. sexdentatus was the aggregation and fusion of the haemocytes to form the capsule about the parasite by the cell membrane breakdown, these findings are supported by Salt (1967) who stated that capsule formation in insects is by cell membrane breakdown and that a syncytium is formed. It is possible that chemical and physiological changes occurred to the haemolymph of the beetle and particularly to the haemocytes in response to the nematode infection. The reaction initiates the aggregation and fusion of other haemocytes. The results of the present study together with all the information about capsule formation, provided some indications to suggest that the haemocytes of the host might exhibit chemoresponse. If this is true, then the stimulus must reach the haemocytes, perhaps this stimulus comes

from the parasite or from substances released by the haemocyte which initiates the reaction. Nappi and Stoffolano, 1971, came to a similar conclusion about the reaction of the larvae of Musca domestica when parasitized by the nematode Heterotylenchus autumnalis.

The presence of the microtubules in the capsules which are originally found in the blood cells especially the amoeboid cells, may be involved in the movement of the cells. Microtubules are known to play a part in cell extension (Byers and Porter, 1964) and they are probably involved in the flattening of the capsule cells.

If the parasite is able to develop in the host and keep abreast with its reactions as in normal hosts, it is not destroyed but simply enclosed in a thin elastic capsule (Poinar, 1969). However, it has been shown by Thompson, (1930) that many insect parasites are ignored by blood cells and these observations led Thompson to a belief that healthy parasites are never attacked, possibly because they do not cause attractive substances to be produced. It can be concluded from the previous observations that the capsules produced by I. sexdentatus against C. diplogaster are either harmless or of definite use to the parasite. There may be a special relationship between the host and the parasite leading to conditions

in which the beetle produces a capsule essential to the parasite for its well-being. This capsule is constructed by the host in a manner that kept the parasites in contact with an oxygen source through tracheal development within the capsule. It would appear that the capsule-forming reaction of the haemocytes has been protective to the host in the first place and this successful parasite has overcome the reaction and even used the capsule for its own purposes. Similar observations and conclusions have been mentioned by Holland (1920) in trematodes when they were encapsulated without any adverse effect, and by Thorpe (1936) when the coccid Saissetia was attacked by the chalid, Encyrtus.

Nuclear membrane and the cytoplasm of the capsule:

The histochemical work on the capsule and its nuclei showed that protein, and DNA were the major constituents of the nucleus. This together with electron-microscope observation for the nuclei, offer a strong means for solving the problem of nuclear fine structure, although it is far more extensive. The combination of these two techniques has been of particular value in answering many questions about the nucleus.

The nuclear membrane is an actively functioning structure (Bernhard, 1959) with pores, nodes and granules.

(Anderson and Beams, 1956; Gay 1956; and Moses 1956). Materials which may have passed through the pores were found as relatively large masses, or as ultramicroscopic particles and as small condensed granules of similar size and density, between the nucleus and nuclear membrane on both sides. Migration of material from the nucleus to the cytoplasm was found in the nurse cells of certain insects (Anderson and Beams, 1956). The capsule nuclei appeared to degenerate, when these electron dense bodies migrated from the nucleus to the cytoplasm. The degeneration of these nuclei is evidence that the nuclear material is extruded from the nucleus to the cytoplasm. Similar observations were obtained by Anderson and Beams (1956) and Bonhag (1955) when masses of granular material were seen to migrate from the nucleus to the cytoplasm of the nurse cells of Rhodnius and the milk weed bug Oncopeltus fasciatus, respectively. Electron micrographs were obtained in which detailed investigations of nuclear organizations in this capsule revealed a mechanism for the exchange of material between nucleus and capsule's cytoplasm.

An explanation must be given for the pocketings of nuclear membrane, and the association which was observed between the cytoplasmic inclusions and these pocketings.

This may be a manifestation of the mechanism for the transport of nuclear material to the cytoplasm. Alternatively the possibility that these pocketings are swellings of the existing membranes concerned their organisation for the passage of the nuclear materials. A similar phenomenon was observed by Gay (1956) in Drosophila salivary gland cell, where the blebbing of the nuclear membrane offers a mechanism whereby nucleocytoplasmic exchanges took place. These blebbings were characteristic features of the cells at the stage of development.

The pocketings of nuclear membranes did not appear during the early stages of the capsule's development (i.e. the capsule from early pupal host) where each nucleus had a relatively smooth membrane, although they were large and amoeboid and the cytoplasm appeared less electron dense. These characters of the nuclear membranes in different stages of development of the capsule suggested that these pocketings were not attributed to the distortion due to fixatives. If that was true the pockets should have appeared either in the early stages of the capsule's development or in later ones, when the nuclei were completely degenerated and the nuclear membrane appeared indistinct. Gay (1956) came to the

same conclusions when he examined the salivary gland cells of Drosophila, using a variety of fixatives.

Deep infoldings of the nuclear membrane were often present particularly in the nuclei of developing capsules which usually contain large abnormal nuclei. Such infoldings increase the nuclear surface, and probably facilitate the passage of nuclear substances. Bernhard (1959) stated that these nuclear membrane enfoldings are characteristic features of rapidly growing cells, especially tumour cells.

Numerous virus infections were found in the abnormal cell by Bernhard (1959); he considered that virus particles are formed within the nucleus and stated that the virus was never present in the nucleus but was in the cytoplasm, and the virus material had penetrated from the cytoplasm to the nucleus and had caused all these alterations. It could be concluded that a functional relationship between the nucleus and the cytoplasm has been demonstrated. This relationship appeared clearly when the capsule's cytoplasm was metabolically active and contained massive mitochondria elements of endoplasmic reticulum, microtubules, membrane limited bodies, probably lysosomes, and golgi bodies. All these organelles were found in the physiologically active

and young capsules which contained active nuclei. The mucopolysaccaride found in the capsule was presumably either secreted by the adult nematode or it may have been derived from golgi vesicles, as suggested by Jones and Northcote (1972). The general appearance in the cytoplasm of the capsule from young adult beetles, of intense metabolic activity is not surprising as the energetic and synthetic needs are for massive transport of nutrients into the cytoplasm and the maintenance of necessary functions, despite a removal of material by the developing nematodes in the capsule. The capsule's cytoplasm consisted of a membrane system which formed the vesicles of a network of tubules through the cytoplasm called endoplasmic reticulum (Palade, 1956). Bernhard (1959) considered the behaviour of these membranes as extremely dynamic within a single cell, according to its age, nutrition and functional state. Palade (1956) stated that endoplasmic reticulum is present in all animal cells and represents important equipment for the organization of such cells and it is greatly affected by, or involved in, the process of cell differentiation.

The dense bodies which appeared in the capsule's cytoplasm were suggested to be lysosome-like bodies but

no histochemical evidence was available to support this suggestion. It has been suggested by Bennett (1956) that the lysosome granules may represent segregated phagocytosed or pinocytosed material and the enzymes associated with the granules may play a role in breaking down some of the contents of the bodies.

In an old capsules from the adult hosts the cellular structure was no longer distinct; all the nuclei had completely degenerated and only fragments of the nuclear membrane remained after nuclear lysis. Whorles of membranes or lamellae were common in these capsules. Swiff (1956) defined them as fenestrate sheets of parallel, membranous elements called annulated lamellae. The same structures have been observed in invertebrate oocytes and they are very common in cancer cells (Swiff 1956). These lamellae are presumably either the remnants of nuclear membrane, remaining from the disintegrated nuclei, or unknown organelles.

Abnormal mitochondria, swollen or with irregular cristae were often found in the capsules. Complete disintegration of the mitochondrial membrane occurred when the mitochondria became condensed and transformed into electron dense dark bodies of globules containing numerous black granules. These damaged mitochondria

have been interpreted as lysosomes (Ashraf et al, 1971). The factors responsible for these changes are not known. Other physiological disorders also resulting from this alteration. Similar changes in the mitochondria were described as lysosomes in the silk worm muscles (Lockshin and Williams, 1965). Ashraf et al (1971) reported similar alteration in the mitochondria of the flight muscles of Scolytus ventralis parasitized by the nematode S. elongatus. They thought that the factors responsible for these alterations were hypoxia and toxins due to the direct invasion of the muscle by the parasite.

The investigation by Ruhm (1956) of genus Contortylenchus, described several new species. Ruhm published the range of body lengths of mature females of C. diplogaster as 1.5 - 2.0 mm, the body width 0.10 - 0.15 mm and a ratio 13.3 - 15.0. While Al-Rabiai (1970) gave a wider range of the body length for her species as 0.880 - 1.930 mm, with a body width range 0.061 - 0.125 mm and an a ratio range of 12.6 - 19.9.

The measurements obtained in this study for mature parasitic females produced a length range between 0.996 - 2.04 mm, 0.041 - 0.192 mm for the body width, and 8.75 - 27.03 for the a value. These measurements fit within the ranges of both Ruhm (1956) and Al-Rabiai (1970). All the

other measurements which were made of free-living stages fitted in with both authors published records. The large variations in the morphometrics of mature parasitic females was related to the density of parasitism. It is believed that the width and the length of the adult nematode varies with the density of parasites within the haemocoel and the nutritional status of the host. The nematode species in this study was thought to be the same species as Al-Rabiai's. The results indicate that this nematode is not a new species as thought (Al-Rabiai, 1970) but C. diplogaster. There is no satisfactory criteria to consider this nematode as a new species, and Al-Rabiai's new species is synonymized with C. diplogaster, (Ruhm, 1956).

Neither Ruhm (1956) nor Al-Rabiai, (1970) had mentioned in their studies from which parasitic densities the nematode measurements were based. These authors did not consider that parasite density would affect parasite morphometrics. These variations are intraspecific rather than interspecific, due to the competition for nutrients when they occur in large numbers in pine bark beetles.

Haemocytes

From this study a number of types of the haemocytes

of I. sexdentatus were described together with their normal and abnormal development.

Although 10 distinct types of haemocytes were described, the prohaemocytes, plasmatocytes, granular haemocytes and amoebocytes were the most dominant. The others were found in low numbers in all beetle instars. Podocytes and vermiform cell were very rare. Prohaemocytes were abundant in numbers in developing instar forms, and were considered as stem cells (Yeager 1945, Jones 1959), from which the plasmatocytes, granular haemocyte and spherule cell are derived. The plasmatocytes develop into granular haemocytes and other types of cell (Yeager 1945 and Jones 1956). They play the major part in capsule formation since their numbers in the haemolymph were greatly reduced when the host was parasitized. They varied in shape and size. This character was used in DHCs where closely related haemocytes were grouped together as plasmatocytes such as amoebocytes, vermiform cells, oncocytoids and podocytes which are all phagocytic cells. More highly vacuolated plasmatocytes were observed in adult beetles than in pupae (these vacuoles correspond to glycogen inclusions (Yeager 1945).)

The granular haemocytes are possibly derived from prohaemocytes, which are regarded as the precursors of

other type of cells. On the other hand they might be derived from plasmatocytes with which they are very similar. These cells were involved in encapsulation suggesting that they are phagocytic cells. These findings agreed with Jones (1956) who suggested that those cells sending pseudopodia are phagocytic as in Sarcophaga, Diptera and Prodenia, Lepidoptera (Yeager, 1945 and Jones 1956). As the nematode is too big to be phagocytozed these cells aggregate around the parasite forming a capsule. Amoebocytes were abundant in the haemolymph of this insect. They were polymorphic and derived from plasmatocytes (Wigglesowrth 1956). The rounded, oval or rod-shaped inclusions of these cells were believed to be mucopolysaccharides (Wigglesworth, 1956). He found these inclusions were strongly positive when stained by PAS. Amoebocytes were active cells and took a major part in capsule formation in I. serdentatus. They collected around the parasite producing PAS positive capsule. If these cells are liberating the mucopolysaccharides as suggested by Wigglesworth (1956), it is possible that melanin formation was prevented by the mucopolysaccharide in the capsule. If the spherule cells and oenocytoids are involved in the capsule, the small spheres from the spherule cells may contain tyrosinase in crystal forms

(Dennell, 1947). Furthermore the oenocytoids contain fine granules or rod-like crystals which may be tyrosine (Riziki 1959, Decleir 1961). Both types of cell were found in the haemolymph but there was no evidence of their involvement in capsule formation, while amoebocytes were definitely involved in the reaction.

Vermiform cells were found in all stages of I. sexdentatus, but they were not seen by other investigators in Orthoptera, Hemiptera or Hymenoptera (Jones 1962) and to my knowledge they were only found in one species of Coleoptera and one Lepidopteran (Jones 1962). Podocytes found in I. sexdentatus are reported from one species of Lepidoptera and Diptera (Jones 1962).

Multinucleate cells were observed in this insect in prohaemocytes, plasmatocytes and amoebocytes. In prohaemocytes, multinucleation is normal (Wigglesworth, 1959). Multinucleate cells in other types of cells rather than prohaemocytes were observed, particularly in infected hosts suggesting that they occur under conditions when these cells became active. Within the capsule, as many as ten nuclei were observed in a single plasmatocyte, suggesting that the nucleus had undergone mitotic division. Ermine (1939) found that in Periplaneta americana and in Tenebrio monitor, prohaemocytes, granular

haemocytes and plasmatocytes were capable of mitotic division under abnormal conditions, while normally only prohaemocytes could divide. These results support Ermine's findings. In Acheta as many as eight nuclei in a single haemocyte have been reported (Cuenot, 1896). Foley and Cheny (1972) pointed out that no multinucleate cells have been observed in the american oyster, Crassostrea virginica, and suggested that these may be occurred under pathological conditions. Wigglesworth (1961) concluded that haemocytes multiply by mitosis throughout the life cycle of the insect and that haemocytes changed their shape when attached to other tissues. Cuenot (1891) considered the haemocytes to be amoeboid only when they were in contact with tissues, but not amoeboid in circulation. The real difficulty in classification was always with cells which were in intermediary form, but the classification given here is adaptable. In the bed bug, haemocytes increase in number enormously during moulting to remove debris (Wigglesworth 1961) and the number of circulating cells vary greatly from time to time. Higher values in THCs were obtained however after injury, in insects attacked by parasites and particularly during ecdysis.

Histopathological changes occurred to the haemocytes due to the presence of Contortylenchus. The qualitative

and quantitative haemocytic changes presented in this study produced several interesting features from THCs and DHCs during parasitism and encapsulation.

In uninfected host the THCs varied throughout a wide range in male and female pupae and adults. It was higher in pupae than that in adults of both sexes. Similar results were obtained by Tauber and Yeager (1936) in Coleoptera, Phyllophaga and Osmoderma when the THCs in the larvae were higher than that in the corresponding adults. Orthopteran nymphs may have slightly higher THCs than adults irrespective of their stage of development (Smith, 1938). It was difficult to compare the THCs and DHCs in I. sexdentatus with any other published account, since results obtained by different techniques are incomparable and techniques may influence the results (Jones, 1962). According to the sexes, the normal THCs in male pupae and adult I. sexdentatus were higher than the females of the two stages, suggesting that these differences may be sex-linked characteristic. In general, variations in THCs due to sexes did not differ significantly, result supported by Wheeler (1961). In Periplaneta americana the adult male had higher and less variable THCs than females (Smith, 1938). On the other hand, the data obtained by

Webley (1951) showed that there were no significant differences between THCs of male and female nymphs, while adult males had significantly higher THCs than females. The THCs of African migratory locusts decrease more in males than in females at the last ecdysis (Webley, 1951). Certain physiological and pathological states, such as oviposition, ecdysis or parasitism could be associated with the changes in THC (Tauber and Yeager, 1934)

In I. sexdentatus the THCs were greatly affected by the presence of the nematode Contortylenchus, and THCs increased after wounding or implantation of foreign bodies in Tenebrio (Reis, 1942). On the other hand, Beard (1940) claimed no changes took place. Because of an increase in pupae the cellular reaction continued after metamorphosis has taken place. The presence of a single parasite, reduced the THCs to 16 and 39 percent in pupal and adult respectively. There were significant differences in the THCs from infected male and female pupae, but not between infected adult males and females. No apparent explanation can be given for these differences. THCs were affected by the density of parasitism whether from pupae or adults.

Higher Contortylenchus infestations caused greater

reductions in the total number of haemocytes present, which suggests that they were either involved in the encapsulation process or that some general phyiological stress created by the presence of the nematodes was responsible for the reduced numbers.

No obvious changes occurred in the THCs of Periplaneta (Day 1952) following massive accumulation of haemocytes due to inury. Rosenberger and Jones (1960) found in Prodenia eridonia that naturally infected larvae had lower THCs suggesting that even the condition of the experiments differ from that of natural infection.

The composition of haemocytes (DHCs) is extremely varied from species to species and within insects of the same species. Certain cell groups may occur in similar proportions (Jones 1962). The haemolymph of I. sexdentatus is composed mainly the same type of cells but these may differ slightly in their quantitative composition if compared with each other. DHCs of parasitized hosts had differed blood pictures from unparasitized hosts. These differences contribute to the evaluation of the defence mechanism of the host when parasitized. Drastic changes in DHCs were found in both infected pupae and adult hosts. Presence of the parasite caused a decrease in plasmatocytes and granular haemocytes, while the prohaem-

ocytes and spherule cell increased in both pupa and adult. A greater reduction occurred in both plasmatocytes and granular haemocytes in adult, proving the continuity of the reaction. Similar responses were obtained by other workers, Wittig (1965) found in Pseudaletia unipuncta great reduction in plasmatocytes especially in the proportion of macroplasmatocytes and an increase in spherule cells and prohaemocytes during acute septicemia. The cells with elongated nuclei which surrounded the necrotic areas were haemocytes involved in encapsulation reaction. Gilliam and Shimahutst (1971) found in the honeybee the count of healthy larvae was higher than those infected by Nosema apis. This destruction of haemocytes would eventually weaken the insect. Nevertheless the blood counts proved a good diagnostic test for infection.

Nappi (1973) reported the DHCs from larvae of Drosophila melanogaster parasitized by Pseudeucoila bochei showed a slight increase in percentage of crystal cells and a decrease in lamellocytes. The parasite is thought to have suppressed the immune reaction of the host. The reaction of Orthellia caesarion against the nematode Heterotylenchus autumnalis is characterized by accumulation and fusion of haemocytes (Nappi 1972).

In the same insects the number of oenocytoids decreased, and these cells are thought to play an important role in encapsulation of the parasite. It was previously believed that phagocytes helped to protect insects against parasites (Thompson, 1930). The effect of the cellular capsule formed by the insect's haemocytes is different for different species of nematode parasites. This study has shown that the aggregated haemocytes which encapsulated the parasites to defend the host was not lethal to the nematodes within the capsule which were able to continue their biological activities. There may be a very complicated relationship between the immune reaction of the host and these parasites.

The investigation revealed a surprising synchrony in the development of the nematode and its surrounding capsule. There was an increase in the volume of the nuclei and DNA which reached a peak in pupal host capsules and subsequently declined in the capsules from adult host. Degeneration of the capsule nuclei appeared to start as the nematodes laid their eggs, and in older capsules the nuclei had disappeared completely. This suggested that the developing nematodes have utilized the contents of these nuclei or been responsible for their

degeneration. The capsule volume increased as the adult nematode grew, and continued to increase to the maximum size when the nematode started reproduction. At a later stage when the nematodes progeny as third stage larvae started leaving the capsule, the capsule volume declined. While some haemocytic capsules formed in other species of insects did not develop beyond a certain stage, the haemocytes of I. sexdentatus continued their development long after the capsule has formed. Their development and that of the encapsulated parasite, appears to be closely synchronised; the nuclei reaching their maximum volume before the nematode becomes reproductively mature. Secondly it is possible that the growing nematode and its progeny stretched the capsule and made it bigger. Decline in the volume of capsules suggested that the reaction has stopped and the contents have been used by the parasites. The number of nuclei in the capsule were reduced in the later stage of development, suggesting that these nuclei were being used by the nematodes. Clumps of feulgen-positive bodies were found in the nuclei, these nuclei derived from haemocytes that had undergone lysis of their cell membrane. Feulgen positive bodies within the nuclei are possibly associated with the rupturing of the nuclear membranes.

Between 250-300 individual nucleated haemocytes were found to compose the entire capsule in pupal beetles. This number may be underestimate of the initial number of haemocytes involved in capsule formation, since there were several difficulties in counting overlapping nuclei, and some haemocytes may have been non-nucleate.

Bird (1961) pointed out that multinucleation in the early stages is related to the syncytium formation, but mitosis may occur occasionally throughout the life of giant cells. Capsule from early pupa was multinucleate due to syncytium formation. Multinucleate haemocytes of the capsule were found, due to mitotic division. Where the cell membranes lyse their content merge to form a dense cytoplasmic matrix. In I. sexdentatus the capsules may have a similar nutritional function to the "nurse" cells of insects. Schrander and Lenchtenberger (1952) stated that in Hemptera "nurse" cells, the cell boundaries become invisible under the microscope due to their break-down, while the nuclei become amoeboid. The cytoplasm of the capsule gradually enlarges as do the nuclei due to their fusion. As the capsule wall becomes thick and the nematodes become surrounded by dense cytoplasm, then number of nuclei declined. The nuclei eventually aggregated in small groups, some of them being considerably

larger than the normal nuclei. This suggested that the nuclear fusion occurs only between opposed nuclei. Similar processes occurred to the cellular capsule.

The nuclear extrusion to the cytoplasm does not usually occur without mechanical difficulty (Schrander and Lenchtenberger 1952). So the Feulgen-positive material gathered around the nuclear membrane, then the nuclei become rounded and finally collapse, releasing their contents into the cytoplasm. It is likely that similar phenomenon occurred to the capsules nuclei. The Feulgen-positive granules in the cytoplasm of the capsule indicate rapid disintegration of the nuclei. It is interesting to know whether the same alteration happens to the normal haemocyte nuclei, or if this is a special modification of the syncytial nuclei. The growing nematodes received soluble contributions from the cytoplasm of the capsule. This comprises not only the cytoplasm liberated by cell membrane breakdown, but may also Feulgen-positive material extruded from the nuclei. These materials according to Schrader and Lenchtenberger (1952) undergo extensive changes before they are incorporated in the growing organism to become Feulgen-negative substances. These changes involve in the formation of polysaccharide and some depolymerization of the deoxyribonucleic acid.

It is not known whether any other stimulus other than the presence of the nematodes as a foreign protein was necessary to stimulate the formation of the capsule. It may be possible that the parasites utilized the haemocytes to grow. They are responsible for stimulating the growth of trachcoles and trachea which ramify the capsule. Tracheation was stimulated by oxygen demand. Oesophageal glands may secrete as they were still appearing in the post-infective female. These glands may produce mucopolysaccharide or enzyme substances as a stimulus which could play an important role in capsule development. The breakdown of cell membranes, mitotic division or protein synthesis could be stimulated by secretion of certain substances by the encapsulated parasites.

In the complex host-parasite relationship between I. seydentatus and Contortylenchus, it is possible that the nematode produced certain substances which caused cells of the haemocytic capsule to fuse. In capsules, especially from pupal hosts, it was found that nuclei aggregated in small groups, the number of nuclei gradually decreased, but each remaining nucleus appeared to be bigger in size, which was probably due to the nuclei fusion.

Roberts et al (1967) showed that the salivary gland cells of the fly larvae Rhynchosciara angelae infected by a microsporidian, became enlarged and the DNA content of their chromosomes become many times more than that of uninfected cells. In later stages of development, when the nematodes third stage larvae started leaving the capsule, the Feulgen-positive granules (DNA) disappeared from the capsule. This suggests that the nucleus originally one of the most important structures in the capsule, playing the main part in protein synthesis through its nucleic acids, had exhausted this role. Caspersson (1950) stated that the nucleus is the important centre for the production of cytoplasmic proteins. Caspersson found that nuclei of younger oocytes were the centres of production of the cytoplasmic protein. While in growing cells, the DNA content of the nucleus appeared to be low in relation to the size of the cells. The cytoplasm surrounding the nuclear membranes is rich with RNA.

Similar results were obtained in capsules from pupae which are regarded as younger where the DNA content was very high, while DNA content was fairly low in physiologically older capsules. The nutritive materials are derived from nuclear DNA as well as the cytoplasm of the capsules.

The present discussion is therefore limited to the description of cellular capsule organelles playing a direct role in nucleic acid and protein synthesis and in cytoplasmic exchanges.

All animal parasitic nematodes which have been studied, live in an environment which is hypertonic to their body fluids (Rogers, 1962; Anya, 1966; Lee, 1965). These nematodes are able to osmoregulate in hypertonic situations, but they are unable to regulate in hypotonic solutions. It has been shown by Stephenson (1942) that water can pass into and out of the body of nematode Rhabditis terrestris because of the effect of osmotic forces. This results in an increase or decrease in the length and size of the body. When this nematode was immersed in distilled water for a considerable time the swelling of the body was reduced and returned to its original length and normal activity. This recovery was because of the existence of active osmotic regulation. The recovery of the treated nematodes could be prevented by injury or a metabolic inhibitor. In 0.85% entomological saline, although it is hypotonic, Contortylenchus first increased in length and began to recover after 90 minutes; this was the only occasion of apparent recovery

possibly because this concentration was very near to its isotonicity which was between 85% and 90% entomological saline. The increase in length may be first caused by absorption of water, because of the permeability of the body wall of this nematode.

When Contortylenchus was immersed in hypertonic solution, the internal osmotic pressure was increased because of the loss of water from the body. After a considerable time the amount of shrinkage was reduced, because after the nematode had lost water, it then took up ions from the surrounding solution followed by the movement of water, which resulted in expansion. Similar results were also obtained for Hammerschmidtiella diesingi when was immersed in hypertonic solutions (Lee, 1960). After 6 hours in hypertonic solution (0.6M NaCl) recovery was completed. When placed in 0.3M NaCl which is normally isotonic they increased in length as if they were in hypotonic solution, suggested that these nematodes had taken up ions when they were in hypertonic solution (after loss of water) increasing the osmotic pressure of their body fluid.

The capsules were exposed to different concentrations of solution and were subjected to two main effects; one caused by the osmoregulation of the encapsulated nematodes

and secondly the other due to concentration of the treatment solution. When placed in 80% seawater, which was equivalent to 0.4M NaCl, and normally hypertonic to the nematode, the water moved from the nematode's body into the capsule, resulting in lower osmotic pressure in the capsule and higher osmotic pressure in the nematode's body fluid. Nematodes lost water, causing them to shrink. The water lost from the nematode and gained by the capsule, causing an increase in size of the capsule. When the nematodes within the capsule began to take up ions followed by water. This resulted again in a higher osmotic pressure of the capsules which caused reduction to their size. The capsules then took up ions, then water and increased in size. No apparent lasting stability was reached in this changeable environment between both the capsule and encapsulated nematodes. It was not possible to run the experiment for a long time because the parasites die after 18-24 hours outside the host. These capsules are living tissues which are able to tolerate osmotic changes in the environment.

The capsules increase in size in hypotonic solution making the capsule nuclei very distinct and enlarged so that they were easy to count.

The capsules were more stable when they immersed in

sea-water, presumably because it is a mixture of salts which is obviously nearer to the composition of the insect haemolymph than a single-salt solution.

The capsules and the nematodes in the insect's haemolymph have to withstand the considerable fluctuations in osmotic pressure which could result from changes in the physiological status of the host; Panikar and Spraston (1941) pointed out that parasites living in the blood and muscles of their host live in more or less osmotically stable surroundings of fairly high concentration.

Insects exposed to a shortage of oxygen will result in an increase of osmotic pressure of the haemolymph which will effect their parasites. The demand that the adult parasitic nematodes make in competition for the available oxygen dissolved in the host's haemolymph are unknown.

It has been suggested (Wigglesworth, 1961) that if the insect struggles violently in the absence of oxygen, increased osmotic pressure of the blood by metabolites set free from the muscles. These changes have important effects on respiration. Release of acid metabolites into the blood when insects are under oxygen stress (Wigglesworth,1961) or other metabolic product from both host and the parasite would lead to immediate changes in the osmotic pressure of the haemolymph. When the blood

pressure of the insects increased, there were lactic acid metabolites present and fluid from tracheal endings removed towards the haemolymph in order to increase the respiratory surface. On the other hand to regulate the increase in osmotic pressure. Insects are able to regulate the osmotic pressure of their body fluid (Wigglesworth, 1961).

Other factors such as the relative humidity may affect the osmotic pressure of the haemolymph as in Tenebrio molitor (Marcuzzi, 1955, 1956) and the accumulation of glycerol in overwintering insects (Asahina et al 1954).

All these factors indirectly affect the parasites that live within the haemolymph. Parasitic females of Contortylenchus diplogaster can withstand changes in osmotic pressure of the order that may occur during moulting and other physiological changes. These findings correspond to the data for other animal parasitic and marine nematodes which have been investigated by other workers (Lee 1960, Anya 1966a, Croll and Viglerchio 1969)

The body wall of the parasitic female Contortylenchus diplogaster differed from closely related entomophilic nematode: Howardula sp. and Deladenus (Riding 1971). Nematodes in these genera lack a cuticle and the body

wall is modified as an absorbent surface for nutrient uptake. Contortylenchus does not have a mouth opening in its anterior cone (Al-Rabiai, 1970) but it certainly feeds, since they increase enormously in size after parasitising the host. The hypertrophy of the hypodermis and the reduction in somatic musculature that were found in the adult parasitic female suggested that these modifications have certain and significant importance. Riding (1971) studied the body wall of adult parasitic Contortylenchus, Howardula sp. and Deladenus and concluded that all were lacking a cuticle and these species with the exception of Contortylenchus have an outer layer of microvilli. The ultrastructural observations on the body wall at different stages of development of Contortylenchus gave strong indications that the body wall must serve not only as a protective covering, but as a metabolically active layer through which nutrients are absorbed.

Contortylenchus lacks a mouth and functional gut; its body wall appeared to be continuous with the hypodermis. The hypodermis contained organelles like mitochondria, golgi bodies, and vesicles, which suggests that it is metabolically active (Anya, 1966).

A membranous layer covering the surface is thrown

into deep surface folds in the adult parasitic nematode.

There are four ways suggested for material to enter the cell (Reâd 1966; Smyth 1969). Through pores, by diffusion; mediated transport; active transport; or by pinocytosis. Contortylenchus has no pore canals like Acanthocephala (Wright and Lumsden, 1969), or microvilli like Howardula and Deladenus (Riding 1971). It has three prominent folds through which most nutrient passage is likely to occur (Al-Rabiai, 1970). Lee (1965) discussed cuticle permeability and stated that when the muscles and hypodermis are scraped away, glucose could pass through the cuticle suggesting that the hypodermis is responsible for selective permeability. The hypodermis is a dense synctium, very osmophilic and containing many protoplasmic inclusions. This region is in intimate contact with the internal tissues and particularly the pseudocoelomic cavity. The hypodermis is of fundamental importance in the transference of essential compounds between the external and the internal nematode. The role of the hypodermis in the transport and nutrition is obviously important.

An extensive investigation has been made of the relationship between Contortylenchus and the beetle's tissues. The membranous layer appeared tubular at higher

magnification and continuous with the nematode surface, suggestive of an early stage in the formation of microvilli.

It is more likely that this membrane separates the nematode's surface from the host haemocytes so it is a protective covering or alternatively if it is continuous with the nematode surface, it may permit and assist the passage of the nutrients through the body wall.

The outer granular layer that has been demonstrated in this study on the outside of the adult parasitic nematode could be an absorptive surface as suggested by Lee (1966) in the case of acanthocephala and cestoda. The outer covering of Contortylenchus may be protecting it from the host's reaction. Mucopolysacchride was reported in the "cuticle" of Contortylenchus in this study. According to Crompton (1963), the host enzymic action could be prevented by mucopolysacchrides. Thus in Contortylenchus it may be responsible for preventing melanin formation in parasitised pupal and adult beetles. Crompton (1963) found mucopolysacchride in the acanthocephalan Polymorphus minutus. Wright (1963) discovered that the intestinal microvilli of Capillaria hepatica were covered with rows of filaments arising from the cell surface and non-acid mucopolysacchride or mucoprotein was present. Moog and

Venger (1952) pointed out that these components may function as substrate for the enzyme alkaline phosphatase. The outer covering protects the parasite by inhibiting the digestive enzymes of the host, (Mowne 1959).

Anya (1966) showed that the inner cortex of Aspiculuris tetraptera, and Syphacia obvelata contained RNA and acid phosphatase and also contained ascorbic acid, which is utilized in collagen formation. He concluded that the cuticle was involved in synthesis of some cuticular proteins. Lee (1962) demonstrated non-specific esterase in the median layer of the cuticle of Ascaris lumbricoides. The cuticle of Metastrongylus sp. contained RNA, non-specific esterase and acid phosphatase activity (Probet, 1969). In Tetradonema plicans, protein was demonstrated in the whole cuticle, mucopolysaccharide in the epicuticle and collagen and bound lipid in the basal region (Hudson, 1972).

In Contortylenchus protein was a general component for the body wall and mucopolysaccharide were demonstrated on the outer layer of the "cuticle" and glycogen, but very slight acid phosphatase activity. The substances not detected, still may have been detected by more sensitive methods. The lateral hypodermal cords were large in both larvae and adult parasitic female. The

tissue was cellular and synthetically active, containing neutral and acidic fats, carbohydrate, protein. Numerous ribosomes and mitochondria probably for enzyme production or protein synthesis. The oesophagus was described by Al-Rabiai (1970) as a cylindrical tube. Two subventral and one dorsal oesophageal glands have also been described (Ruhm 1956; Al-Rabiai 1970) from light microscope studies.

The results obtained in this study for the oesophageal region with electronmicroscope showed that these glands actively synthesized mucopolysaccharide granules, which was similar to the companion cells in T. plicans (Hudson, 1972).

An obvious similarity was apparent between the oesophageal glands of Contortylenchus and that of other nematodes in general function and cytoplasmic inclusions, like the dorsal glands of Meloidogyne javanica (Bird, 1971). Jennings and Colam (1970) found that the dorsal and one of the sub-ventral glands in Pontonema vulgaris were acidophilic and the other sub-ventral was basophilic. Contortylenchus oesophageal glands were nearly similar in structure and possibly function to tetrad cells of T. plicans (Hudson, 1972) or to the sub-ventral glands of Nippostrongylus brasiliensis (Lee, 1970).

Lee (1970) stated that the sub-ventral glands of

N. brasiliensis were secretory rather than excretory and are probably involved in feeding by producing histolytic secretions. Enzymes were located in the secretory granules. The oesophageal glands of Contortylenchus are probably involved in feeding mechanisms and probably they secreted digestive enzymes or other substances as mucopolysaccharide which may pass through to the outside to form a protective layer around the nematode.

Hudson (1972) listed a number of functions for the companion cells of T. plicans; enzymes production, production of mucopolysaccharide as storage product for eggs, storage of excretory products, production of materials involved in host penetration, and secretion of a protective layer. Contortylenchus females have no mouth, no anus, no excretory system or other apertures other than the reproductive tract opening. It is more likely that these glands could be involved in storage of the excretory products. It would be of interest to study the development of these glands in the pre-infective female and to observe the possible changes that occur after the nematode had penetrated its host.

Nuclei, mitochondria, golgi bodies, canaliculi and cuticularised duct were present in the oesophageal

and gland regions. These glands were probably involved in synthesis of enzymes and structural proteins. Absorption of nutrients via canaliculi could be the possible alternative function of these glands, since Contortylenchus has no functional gut. In Trichinella spiralis Bruce (1970a) found that the oesophageal tissue was cellular and contained secretory granules, glycogen and mitochondria which is the case in Contortylenchus. An absorptive function for tetrad cells in T. plicans has been suggested by Hudson (1972).

SUMMARY

1. Contortylenchus diplogaster nematode parasite of bark-boring beetles Ips sexdentatus (Boern), exposed to haemocytic reaction in the host haemolymph.

2. I. sexdentatus were collected from Pinus sylvestris and reared successfully in the laboratory in well ventilated logs.

3. The life-cycle of the nematode is synchronised with the life-cycle of the host.

   All the Contortylenchus found parasitizing the pupal stage of the host were completely encapsulated but the extent of encapsulation decreased in relation to parasite density in the adult host.

4. The nematode species in this study was thought to be the same experimental animal as Al-Rabiai's (1970). However, the result obtained indicates that this nematode was indeed C. diplogaster, (Ruhm). The morphometric variations are considered to be intra-specific rather than interspecific.

5. A complicated relationship between the immune reaction of the host and the parasites leading to the condition in which the host produced a capsule through which active or passive transport of soluble nutrient takes place. These nutrients are essential to the develop-

ment of the parasites. The capsule formation reaction have been protective to the host, while the well adapted parasite C. diplogaster utilizes this capsule for their development.

6. The immune reaction of pupae and adults of I. sexdentatus against C. diplogaster is characterised by the accummulation and fusion of haemocytes to form a homogeneous, cellular multinucleate capsule around the parasites. Secondly, proliferation of the tracheoles and tracheae was associated with encapsulation. The encapsulated nematodes developed and reproduced successfully in I. sexdentatus haemolymph.

7. The various classes of haemocytes present in all stages of the life-cycle of I. sexdentatus have been studied, classified, totally and differentially counted. Ten types of haemocytes were identified as follows: Prohaemocytes, plasmatocytes, granular haemocytes, vermiform cells, multiramous vermiform, podocytes, amoebocytes, spheroidocytes, oenocytoide and eruptive cells.

Abnormal and pathological changes in I. sexdentatus haemocytes result from defence reactions against the nematode C. diplogaster infection.

Observations on the histological changes (DHCs) and

(THCs) were made. THCs were decreased as the parasite burden increased. The effects on the THCs and DHCs after parasitization were studied.

8. The ultrastructure and the histochemistry of the capsules have been examined and studied after the initiation of the reaction until the nematode progeny had departed from the capsules. Capsules were formed within the first day of pupation (containing completely encapsulated nematode).

The cell walls (haemocytes) accompanied this reaction breakdown causing thickening of the capsule wall and increasing density of the cytoplasmic area.

The nuclei first increased in number and reached a peak at the time the nematode commenced egg production. The nuclei are large amoeboid in shape, and dense electron bodies were found along the nuclear membrane. The Feulgen positive bodies in the capsules decreased as the nematode developed. After the nematode commenced egg laying the numbers of nuclei declined. The capsule wall contained proteins, mucopolysaccharide, glycogen and the cytoplasm was rich with mucopolysaccharide and protein.

9.a. Females of C. diplogaster live in the haemolymph of I. sexdentatus in hypertonic media

9.b. They recover their normal length after a period of shrinkage within 18-20 hrs. in 0.4, 0.5, 0.6, 0.8 and 1.0 M of sodium chloride, but not in hypotonic solutions or in hypertonic sucrose.

9.c. Females which have regained their original length in hypertonic solution, increased in length or swell when placed in normal isotonic solutions (0.3 M NaCl).

9.d. Capsules surrounding the nematode can osmoregulate well in hypertonic solutions of NaCl and sea water and they were more stable in sea water.

10. Multiple infections reduce the size and fecundity of C. diplogaster.

11. The parasite reduces and destroys the fat body and membranes of I. sexdentatus and reduces the egg output and retards the host's development.

12. The ultrastructure of the body wall of developing adult female C. diplogaster studied. It has no normal cuticle and a membranous covering is present which seems to be continuous with the hypodermis.

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APPENDIX

Appendix 1

Diluting fluid used for the haemolymph (Weir, 1964).

Table 1

Constituent	Concentration
NaCl	0.081 M
KCl	0.002 M
$CaCl_2$	0.001 M
Gention violet	0.005%
Glacial Acetic Acid	0.125%

Appendix 2

Calculation of number of haemocytes in one cubic millimetre of haemolymph.

Each square in the ruled area of the slide is 1.0 x 1.0 mm, the depth is 0.1 mm.

So $1.0 \times 1.0 \times 0.1 = 0.1\ mm^3$.

The numbers of cells observed were multiplied by 10 to give number of blood cell in 1 mm^3 in the diluted blood. ($1.0 \times 1.0 \times 10 \times 0.1 = 1\ mm^3$). By multiplying the result by the diluting factor, the number of blood cells per 1 mm^3 of pure blood was obtained.

Appendix 3

Description of the constant pressure respirometer (Spencer Davies, 1966)

The respirometer is composed of the respiration chamber which is screwed into a baseplate and connected to a similar compensation chamber by tubes and a manometer capillary, a manometer block and lastly a micrometer device mounted in the manometer block and connected with the respiration chamber.

Two small tubes contained a piece of cotton wool saturated with 20% potassium hydroxide inside a roll of filter paper also wet with the same solution (KOH). These tubes were fixed with celotape on the side of each chamber and used for absorption of CO_2 which is liberating during the respiration process. A wet piece of filter paper was placed in the bottom of each chamber to ensure rapid saturation of the respiratory atmosphere, then the respirometer was mounted in a constant temperature water bath at 20.4°C. Since the air was saturated with KOH, no CO_2 was present until the insect exhaled; CO_2 replaced the O_2 inhaled by the insect is absorbed. Thus the respiration rate is recorded by the movement of the manometer fluid. To calculate the O_2 volume used by the insect during known period of time, the diameter of the micrometer spindle was measured (6.7945 mm) from which the radius was calculated (3.3972 mm). The volume of oxygen used by each insect was calculated by multiplying the height of oxygen by the surface

area of the spindle.

The equation:- $v = \pi r^2.h$

v = volume, r = radius h = height of which the manometer fluid moved.

Appendix 4

Determination of oxygen consumption in $\mu l O_2/\mu g$ d.w./h. The dry weight was obtained from the differences between the two pans of the microelectron pan balance, one of these used as a standard and the other contained the dried nematodes.

Example

40 nematodes were used.

The weight of empty pan = 0.0042 mg.

The weight of the pan and the nematode = 0.1903 mg

So:

0.1903 - 0.0042 = 0.1861 mg the dry weight of the nematode.

The amount of O_2 dissolved per 1 ml of entomological saline under 1 atmospheric pressure at 30°C is 0.0260 ml. Four ml. of saline were used so 0.0260 x 4 = 0.1040 ml O_2 in 4 ml saline. 8% of the oxygen was used by the nematodes in 15 minutes.

$\frac{0.1040 \times 8}{100}$ = 0.00832 ml of O_2 was consumed in 15 minutes by the nematode.

The O_2 consumed in 1 hour = 0.0332

to express per 1g dry weight/hr.

$$\frac{0.0332 \times 1000}{0.1861} = 178.398 \text{ ml } O_2/\text{g.d.wt./hr.}$$

to express in $\mu l O_2/\mu g$ d.wt./hr.

178.398 ml O_2 utilized in 1 hr/1g d.wt.

178.398 x 1000 $\mu l O_2$ - 1 x 1000 x 1000 μg.d.wt./hr.

178398 $\mu l O_2$ - 1,000,000 ug. d.wt./hr.

0.178 $\mu l O_2$ - 1 μg d.wt./h.

Therefore 0.178 $\mu l O_2$ used by each μg of dry weight in one hour.

Appendix Table 2 Parasite Morphometrics and Multiple Infections

No. of parasites/host	No. of nematodes measured	Body Length (mm)		Body Width (mm)		a Value		Mean volume (mm^3)	Percentage reduction in volume	S.E.
		Range	Mean	Range	Mean	Range	Mean			
1	10	1.62-2.04	1.89	0.108-0.192	0.146	8.75-18.88	12.526	1.129	-	-
2	20	1.59-1.94	1.733	0.095-0.16	0.137	9.9-17.3	13.38	1.029	8.45	± 2.33
3	27	1.46-1.80	1.41	0.054-0.168	0.115	12.0-27.0	13.9	0.837	25.37	± 4.08
4	36	1.33-1.7	1.545	0.063-0.10	0.096	13.4-25.8	17.8	0.914	18.51	2.33
5	35	1.32-1.56	1.37	0.080-0.12	0.100	9.7-16.25	14.03	0.817	27.3	2.38
6	42		1.35		0.085	13.6-18.25	15.9	0.799	28.9	1.4
7	42		1.20		0.079	12.5-22.5	17.23	0.712	36.05	3.48
8	40	1.02-1.2	1.25	0.060-0.074	0.075	17.02-20.6	19.16	0.731	34.25	± 2.22
10	40	0.99-1.04	1.07	0.041-0.055	0.072	18.4-25.0	21.88	0.637	43.3	± 3.03

Appendix Table 3 Decrease in Parasite Fecundity in Multiple Infections

Numbers of parasites/ host	Number of Replicates	Number of progeny per parasite Range	Mean No. of progeny/ parasite	Percentage reduction
1	10	172 - 1805	821.5	-
2	9	105 - 870.5	463.3	43.6
3	9	147 - 1002	451.03	45.0
4	6	160 - 715	378.0	54.0
5	6	511 - 171.2	308.9	62.2
6	6	306 - 143	258.2	68.5
7	5	110 - 263.4	168.1	79.5
8	5	124.5 - 232	213.8	73.6
10	5	99.6 - 304.2	166.3	79.7

Appendix Table 4

Decrease in Parasite Fecundity in Multiple Infection

Equation	b = 5.126	Value of (t) (d.f)	P
$y = a + bx$	r = .9426	6.916 (6.d.f.)	0.001
$x = 2$	t for (6.d.f.) = 6.916 significant		
$y_1 = 44.67$	for P 0.001		
$x = 8$	Therefore t value is significant since		
$y_2 = 75.43$	the 0.1% critical value of t for (6.d.f.) is 5.959.		

Appendix 5

THCs in Multiple infection. Test for Significance

Students 't' test was used

$$t_{\text{degrees of freedom}} = \frac{x_1 - x_2}{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$$

1. For adult males and females

adult male 5.929, $Sn^2 = .9754^2 = .9541$

" female 4.739, " $= .3598^2 = .1295$

Combined variances = 1.0396

$$t = \frac{5.929 - 4.739}{\sqrt{.9541 + .1295}}$$

$$= \frac{1.190}{1.0396}$$

$$= 1.144$$ not significant

so they were combined in one line, as shown in the table.

Equation $y = a + bx$	b	Value of F(d.f)	Probability
when $x = 1$ $y_1 = 45.332$	5.33	85.1 (1,12)	significant value for
when $x = 5$ $y_2 = 66.64$	r = .9361		P = 0.05

Therefore F value is significant since the 5% critical value for F (1,12) is 4.75.

2. For pupae, males and females

male pupae

$b = 3.49836$, $Sb^2 = (.187038)^2 = .03498$

female pupae

$b = 2.43197$, $Sb^2 = (.30026)^2 = .090156$

$$t = \frac{3.49836 - 2.43197}{\sqrt{.03498 + .090156}} = \frac{1.06639}{.35375}$$

$$= 3.0145$$

$P = 2\% > (+) 1\%$

Therefore it was not justified in combining males and females pupae in one line.

Male pupae

Equation $y = a + bx$	$b = 3.498$	Value of F(d.f.)	Probability
when $x = 1$ $y_1 = 16.961$ when $x = 6$ $y_2 = 34.451$	$r = .993$	349.8 (1,5)	$P = 0.05$

$F(1,5) = 349.8$ significant value for $P = 0.05$

Therefore, F value is significant since the 5% critical value for (1,5) is 230.2

Female pupae

Equation $y = a + bx$	$b = 2.431$	Value of F(d.f)	Probability
when $x = 1$ $y_1 = 19.08$ when $x = 8$ $y_2 = 36.10$	$r = .963$	65.60 (1,5)	$P. = 0.05$

$F(1,5) = 65.60$ significant value for $P = 0.05$

Therefore, F value is significant since the 5% critical value for F (1,5) is 6.61.

Appendix 6

The effect of parasite density on the extent of the encapsulation.

Equation $y = a + bx$ when $x = 2$ $y_1 = 43.95$ when $x = 8$ $y_2 = 20.943$	$b = 3.8345$ $r \quad .92^{74}$	Value of $\underline{t}$ (d.f.) - 6.808 (8d.f.) $P = 0.001$ value of t is significant for (8d.f.) since the absolute value for 0.1 % is $\underline{5.041}$