DISCUSSION
IN VITRO INCUBATION

The present experiment clearly shows that Nemaetur has direct effect on larvae of *H. incognita*, such as change in shape, movement, and other symptoms indicative of poisoning of nervous system and interruption of muscular activity.

Hotsinger (1961), Kondrollochis *et al.* (1970), Nelmes (1970) and Nelmes & Keerweeven (1970) considered that organophosphate and carbamate nematicides caused treated nematodes to become inactive rather than killing them directly. Nelmes *et al.* (1973) suggested that direct contact of nematodes with organophosphate or carbamate nematicides, or their metabolites, in soil or plants, may influence both the behaviour and development of the nematode. Further, nematicide solutions were shown by Staniland & Stone (1953), Nelmes (1970), Evans & Thompson (1971) and Kondrollochis (1971) to affect the posture of nematodes.

In the present studies, the motility of *H. incognita* larvae incubated in Nemaetur solution is decreased with increased concentration. The recovery of Nemaetur treated larvae is dependent on treatment concentration and exposure period, since larval recovery decreased as concentration increased. In high concentrations larvae become paralysed.
in short exposures and seem to be dead. At low concentrations and short time exposures reaction is reversible. The change in movement and shape, decreased body undulations and abnormal stylet movement of *M. incognita* larvae is in conformity with the observations of other workers. 

Keetch's *in vitro* studies (1974) on the effect of aldicarb and phorate on adult female *Aphelenchus avenae* indicate that there is no mortality over a 24 hour period in solutions of 25 ppm aldicarb or 23 ppm phorate; abnormal stylet and median bulb activity, the adoption of coiled postures and a gradual decrease in the rate of dispersal was observed.

This is similar to the observations made by Oliff (1965) and Kondrollochis (1971) for nematodes exposed to varying concentration of thionacin. When the coiled *A. avenae* were allowed to recover in water for a period of 24 hours, their powers of dispersal were found to be unimpaired by exposure to the nematicide solution indicating that these compounds (aldicarb and phorate) have a narcotic or paralyzing rather than a lethal effect on the nematodes (Keetch, 1974).

McLeod & Khair (1975) observed that aldicarb, up to 8 ppm, had a small effect against egg hatch which agrees with the view of Berg & Cuany (1972) that in the field it acts against phases of the life cycle other than on hatching.
The resumption of hatching, when egg masses are removed from oximecarbamate nematicide or organophosphate nematicide, has already been reported (Sasser, 1952; Matsinger, 1961; Barge & Cuany, 1972).

McLeod & Khair (1975) observed resumption of hatching in Meloidogyne spp. after removal from some oximecarbamate and organophosphate nematicides. They suggest that these nematicides under in vitro conditions do not kill plant parasitic nematodes at concentrations well above those used in the field. Paralysis, however, occurs which persists up to 24 hours "after dilution of the nematicides" and that the chief effect of these nematicides may be systemic action against nematode development with in the root.

Greco & Thompson (1980) observed that at certain concentration (0.48 µg/ml for M. javanica and 4.8 µg/ml for H. aubactii) the effect of phenamiphos on eggs is largely nematostatic and some eggs resume hatching once the nematicide has either degraded or flushed away. Higher concentrations are apparently lethal.

Morban & Viglierchio (1980) in their experiment with Pratylenchus vulnus observed many similarities in symptoms (irritability, lack of co-ordination, convulsions, contractile paralysis and death) after treatment with phenamiphos and carbofuran with those observed with insects
and vertebrates treated with cholinesterase inhibitors which support the hypothesis that these nematicides act by interfering with the transmission of nerve impulses to somatic musculature. However, immobile *P. vulnus*, with the exception of those individuals exposed to higher doses, continued to manifest internal organ activity (pulsation of the median bulb, stylet movement, vaginal vulval contraction and spicular movement) for extended periods during continuous exposure to these chemicals. This behaviour was observed with nematicide concentration of both carbofuran and phenamiphos 100 times the amount necessary to inhibit somatic musculature activity (Narban & Viglierchio, l.c.).

Croll (1975) observed similar behavioural responses with other nematodes treated with biologically active substances and explained the difference in two kinds of muscular activity by hypothesizing two different chemical nerve ring co-ordinating system, an exogenous system co-ordinated by acetylcholine, controlling primary somatic musculature and an endogenous system possibly co-ordinated by serotonin or epinephrine, controlling the musculature of internal organs.

Data from present tests indicate that *H. incognita* larvae treated with Nemacur at low concentrations (up to 1000 ppm) and shorter time period regain activity if allowed to recover in distilled water for 24 hours, whereas with
the higher doses (2500 ppm and above) treated larvae show negligible or no recovery in the same period. The recovery experiments show that the effects of short exposures with low concentration in *Nemacur* are reversible with *X. innocita* larvae, as the latter show movement on touch but in long exposures with low concentrations the larvae do not recover. This also shows that *Nemacur* in water has a long life and results in irreversible poisoning of larvae.

Morben & Viglierchio (1980) observed similar recovery behaviour when they transferred *Pratylenchus vulnus* to aerated distilled water after treatment. The reversibility of the reaction disappeared with increasing concentration of nematicides. The disruption of dispersion pattern of *P. vulnus* at low doses or time exposure suggests that these chemicals have the potential to change the normal response behaviour of nematodes possibly by impairing a nervous system co-ordinating function (Morben & Viglierchio, 1980). This is consistent with the generally accepted notion that most carbamate and organophosphate have little or no direct killing effect on nematodes at concentrations achieved in commercial field application (Hough & Thompson, 1975; Kondrollochis, 1972; Nelmes et al., 1973).

The mode of action of organophosphates on insects and mammals is already known where they inhibit acetylcholinesterase. From the present observations it is presumed
that their main effect on nematodes, too, is acetylcholinesterase inhibition. It seems, however, that the enzymes involved and/or the muscular tissue they act upon are less essential in nematodes than in insects and mammals, because nematodes are killed much more slowly by organophosphates. Unlike the muscles of most animals, those of nematodes are not innervated by motor nerves but by processes which pass from the muscles to the nerve and not by nerves sending their fibers to the muscles (del Castillo, 1969). Perhaps, this could be because of the lack of circulatory system and well developed musculature in nematodes. It may be possible that some enzymes other than those studied in insects and mammals are involved because organophosphate systemics inhibit a wide range of enzymes (Ooms, 1961).
CUTICLE

HETOLOGY

The nematode cuticle shows great structural diversity in different families and there are often differences, not only between the sexes within a single species, but in thickness and sometimes structure in different regions of the same individual, as also observed in the present investigation, where cuticle is the thickest in the perineal region and thinnest in the cephalic region.

Chitwood & Chitwood (1950) gave a comprehensive review of the cuticle of nematodes. The structure of nematode cuticle had been reviewed by Inglis (1964) and Lee (1966b). Comparative studies with the light microscope have shown that there is a considerable variation in the number and types of layers of the cuticle in different genera of nematodes (Chitwood & Chitwood, 1950). However, these may be grouped into a basic pattern based on the results of histochemical analysis of the cuticles of a number of widely divergent species (Bird, 1957; Monne, 1955, 1959 & 1960).

The terms used for the various layers of the nematode cuticle were first introduced by Van Bommel (1895). Recently (1980) a modified basic nomenclature of nematode cuticle
has been proposed by Bird.

Most modern authors agree that the nematode cuticle appears to consist of or to have evolved from, a three layered or three zoned structure (Bird, 1971; Dick & Wright, 1973; Johnson et al., 1970; Lee & Atkinson, 1976) and *Meloidogyne* fits into this pattern.

The present investigations reveal that the structure of cuticle of *Meloidogyne incognita* is the same as in case of *H. javanica* (Bird & Rogers, 1965a). Cuticle here consists of an outer external cortical layer (epicuticle), internal cortical layer (cortical layer) and a thick fibre layer (basal layer) which appears to be two layered and merges with the hypodermis and is not separated from it by basal lamella (nomencature of layers of cuticle, given in parentheses, is after Bird, 1980). In *Acanthoscelides* a layer known as basal lamella separates the fibre layer from the hypodermis (Bird & Bird, 1969). It consists of fine fibrils similar to those of the fibre layers (Hins, 1963; Watson, 1965b) and it merges with the hypodermis beneath. However, Watson (1965b) reports the presence of a plasma membrane in *A. lumbricoides* which separates the basal lamella from the hypodermis. Similarly in *Xiphinema index* (Roggen et al., 1967) and the larvae of *Hypocrestrongylus brasiliensis* (Lee, 1966a) the cuticle is separated from the hypodermis by a plasma membrane. However, similar structures
in *Parasarcia anuorum* are thought by Hins (1963) to be membranes of the hypodermal endoplasmic reticum.

**HISTOCHEMISTRY**

The present histochemical studies reveal that the chemical composition of the cuticle of *Meloidogyne incognita* is similar in many aspects to that of the cuticle of other nematodes which have been studied (Bird & Rogers, 1956; Bird, 1957).

The present observations reveal that essentially proteins and lipids account for the chemical make up of the cuticle as stated by Chitwood (1938), Trim (1949), Bird (1957) and Lee (1962b) with traces of carbohydrates as also reported by Bird (1957), Bird & Rogers (1956), Beam (1964) and Anya (1964a & 1966a) in other nematodes.

A possible presence of neutral polysaccharides was also confirmed by a general pink colouration imparted to the cuticle of *Phocanema decipiens* by PAS and Best's carmine reactions (Kan & Davey, 1968). In *Aspiculuris tetraptera* a very weak reaction was obtained with Best's carmine in the cortex of the cuticle (Anya, 1964a). The fact that in *A. tetraptera*, Best's carmine, which is fairly specific for glycogen, showed a better reaction than PAS in the
cortex suggests that a polysaccharide, similar to, if not identical with, glycogen is present in this layer in addition to lipids.

The histochemical test for acid mucopolysaccharides is generally positive in the cuticle of *Meloidogyne*. The presence of acid mucopolysaccharides in the cuticle also appears to be of significance as the acid mucopolysaccharides regulate the permeability of the cuticle (Wright, 1968). Collagen always occur in association with hyaluronic acid and chondroitin-sulphate containing-acid mucopolysaccharides (Gross et al., 1958; Harkness, 1961). It may be possible that in *Meloidogyne*, at least part of the PAS positive and metachromatic substances in the layers of the cuticle, belong to this class of carbohydrates: a result that is also suggested by studies of Ascaris cuticle (Magasawa, 1961). Clarke (1968 & 1970) expressed doubt regarding the presence of acid mucopolysaccharides in the cuticle. Sood & Kalra (1977) observed PAS positive material, apparently acid mucopolysaccharides, in the cuticle of *Xiphinema insigne*.

On the other hand, distinct β-metachromasia with toluidine blue is demonstrable in the present cuticle. Metachromasia is one of the properties of acid mucopolysaccharides. Kan & Davey (1968), in their studies with
Phocanema decipiens, observed the failure of hyaluronidase to digest the layers of cuticle which indicates that intense metachromasia is not so much an indication of the occurrence of acid mucopolysaccharides as it is a reflection of the predominance of acidic groups in the bands of matrix layer of Phocanema.

A light staining after PAS and Best's carmine tests in the cortical and basal layers here in the cuticle of *M. inconspicua* confirms the presence of carbohydrates in traces. This is in accordance with the findings of earlier workers. The presence of polysaccharides (both neutral and acidic) here in the cuticle of *M. inconspicua* can be attributed to the same function as proposed by Wright (1968) who observed that cuticle of *Trichuris avocastoria* was less permeable to large molecules than that of *Capillaria hepatica*. He attributed this difference in permeability to the presence of more polysaccharides component in the matrix layer of *Trichuris avocastoria* than in the *Capillaria hepatica*. He concluded "It seems reasonable to propose that the polysaccharide-protein content of the cuticle may be responsible for regulating the permeability of the body wall."

Pease (1966) has drawn attention of cytologists to the importance of polysaccharides and polysaccharide-protein complexes of surface coats of epithelial cells. The
properties of such polysaccharides, as reviewed by Pease (1966) and Schubert (1964), suggest that these should also serve admirably to regulate the permeability of nematode cuticle. Thus water, ions and small molecules would freely enter while larger molecules would be excluded.

Due to the presence of acid mucopolysaccharides the cuticle of *M. incognita* may act as physiologically active absorptive surface as proposed by Monne (1959). Monne (1959) postulated that the presence of acid mucopolysaccharides in the Acanthocephalan epicuticle serve to shield enteric helminths against the host digestive enzymes where acid mucopolysaccharides or glycoproteins are intimately associated with a physiologically active absorptive surface.

A relationship between ion accumulation and the acid mucopolysaccharides of surface and intracellular cytomembranes has been indicated by the work of Philpott (1964) and Morard (1967). Katchalsky (1964) suggested that acidic polypeptides, mucopolysaccharides and glycoproteins may act as biological cation exchange resins in Acanthocephala. With the acid haematin test and the test employing the Sudan stains, a positive reaction followed by a negative reaction after pyridine extraction indicates the presence of lipid in the cuticle in the present studies. While elements in the cuticle of *M. incognita* sometimes stained faintly with these reagents, the reaction was
unaffected by pyridine extraction.

Earlier attempts (Bird, 1957; Anya, 1966a; Ken & Davey, 1968) for the demonstration of lipids were unsatisfactory as these were bound to proteins in the outer cortex which resist pyridine extraction and stain intensely after their unmasking with acetic acid. According to Baker (1946) those substances which continue to stain with the acid haematin or Sudan dyes after pyridine extraction may be either phospholipids which are so firmly bound to a protein as to resist pyridine extraction, or a substance which, while soluble in pyridine in the pure state, becomes insoluble under the circumstances in which it occurs in fixed tissue. In view of their resistance to pyridine extraction, any lipids which do occur are probably bound to the structural protein. In the present study a positive test after Berenbaum's technique confirms the presence of bound lipids as lipoproteins in the cuticle of H. inermis.

Other workers experienced similar difficulties with lipids in nematode cuticles. Anya (1964a) showed the presence of a thin layer of lipids beneath the cortex of Enterobius vermicularis and Aepiculiria tetraptera. He also demonstrated the presence of cortical lipids in these nematodes by means of Sudan black B and the Luxol fast blue procedure for phospholipids. Later Anya (1966a) showed that this staining was resistant to extraction with pyridine.
Lee (1960), in studies on Thalastoma bulhossi and Hippocampus brasiliensis, using a variety of stains, failed to reveal lipids and Von Keesmitz (1912) found none in A. lumbricoides. Though Rogers & Lazarus (1949) and Fairbairn (1955a) found small but significant amounts of phospholipids in the cuticle of A. lumbricoides.

Anya (1964a) concluded that distribution of phospholipids in A. tetraptera and E. vermicularis is mainly in tissues with a structural function, an observation in accord with Fairbairn (1960) who stated "It is probably safe to assume that as in other animals, the phospholipids and sterols are structural components of cells, concerned in the formation of "cellular and subcellular membranes".

Sood & Kalra (1977) have detected lipid in the outermost layer of cuticle of Hapalochlaena contortus and Xiphineus insignis. Lipids, according to these authors, may provide structural stability by forming strong lipoprotein complexes, which, in turn, make the cuticle resistant to harmful substances in their environment. The same function can be assigned to the lipoproteinaceous cuticle of M. inconnotata in the present studies.

The cuticle of M. inconnotata, here, shows the general presence of proteins as confirmed by a positive mercury bromophenol blue, Himes & Moriber's and CTZ tests. The
epicuticle and cortical layer differ in their chemical nature because disulphide (SS) groups of proteins are peculiar to the epicuticle and are probably associated with its resistant properties. Sulphydryl (SH) groups are found both in epicuticle and cortical layers. Presence of arginine in good amount, histidine, lysine, cysteine and traces of tyrosine is also confirmed by CTZ and its controls.

Hydroxyproline has been reported in traces in the cuticle of *Meloidogyne* (Bird, 1958b). The small amounts of aromatic sulphur containing amino acids and the presence of hydroxyproline in *Heterodera rostochiensis* (*Globodera rostochiensis*) egg shell suggests that the egg shell protein is collagen (Clarke et al., 1967).

The cuticle of *Ascaris lumbricoides, Toxocara cati* and *Strongylus equinus* contains majority of naturally occurring amino acids which are similar in number and type albeit with a slight quantitative difference; the most noticeable of these is the greater concentration of hydroxyproline in the cuticle of *Strongylus equinus* as compared with the amounts of both ascaroids.

Different authors have assigned different functions and nature of the nematode cuticle on the basis of various types of protein present in it. There has been considerable discussion on the identity of some of the structural proteins
of the cuticle of nematodes (Magath, 1919; Mueller, 1929; Chitwood, 1936 & 1938; Faure-Fremiet & Garrault, 1944; Picken et al., 1947; Watson & Sylvester, 1959; Bird & Bird, 1969).

The epicuticle of *Ascaris*, because of its sulphur contents (Flury, 1912; Chitwood, 1936) and histidine-lysine-arginine ratio (Savel, 1955), has been considered to be a keratin. Further evidence for keratin has been provided by the histochemical detection of disulphide and sulphhydryl groups in this layer (Carbonell & Apitz, 1961; Anya, 1966a). The presence of keratinous protein in the cortical layers and collagenous protein in the fibre layers in *Haemonchus contortus* was also reported by Sood & Kalra (1977).

On the basis of the presence of sulphur contents (SS and SH groups), vide supra, in the epicuticle of *M. incognita*, cuticle here could be considered as keratin. However, the external cortical layer of cuticle of *A. lumbricoides* gives a collagen type X-ray diffraction pattern (Faure-Fremiet & Garrault, 1944), is soluble in hot dilute alkali (Fairbairn, 1957) and is not completely dissolved by 0.5% thioglycollate (Bird, 1957; Anya, 1966a) shows that it cannot be a keratin. Same results obtained by Bird (1958b) in case of *M. javanica* and *M. haemol* lend support to the view that just on the basis of sulphur contents, here, in the cuticle of *M. incognita* it cannot
be considered a keratin. Further, according to Brown (1950) keratin does not occur in invertebrates and that this group of animals has developed a collagenous type of exoskeleton which may be rendered more stable and resistant by tanning.

Apparently, the cuticle of nematodes is composed of secreted collagen associated with hyaluronic acid, chondroitin-sulphate containing-acid mucopolysaccharides and a small amount of lipid (Lee, 1966b). The collagen in nematodes is usually present as fibrils which are more numerous and more closely associated with each other in some layers (the cortex and fibre layers) than in others (the matrix layer).

The protein of the cuticle of Ascaris and earthworms from its origin, might be regarded as a "secreted collagen" (Picken et al., 1947; Reed & Rudall, 1948).

Bird (1958b), on the basis of his studies concluded that chemical composition of the egg sac and cuticle of Heliodonysa sp. probably seems to resemble the cuticle of other nematodes which have been studied in that they resemble secreted collagen in their reactions to various reagents.

It may be stated that secreted collagen differ from normal collagen in that they show no trace of long periodicities, either in X-ray patterns or in electron
Evidence has been accumulating in recent years, however, that not all the proteins which, on chemical or other physical grounds are regarded as collagen show this periodicity (Rudall, 1955; Gross et al., 1956; Watson, 1958). Moreover, the studies of Schmitt et al. (1955) indicate that axial periodicity in collagen fibers is a reflection of the mode of molecular aggregation of the protofibrils, a phenomenon which is, to a large extent, governed by the environment. Thus periodicity in structure is not necessarily a fundamental criterion on which a categorical classification of a structural protein as collagen can be based (Harkness, 1961; Harrington & Von Hippel, 1961).

Ellenby (1946) found the cyst wall of Heterodera, which is the adult cuticle, to be quinone tanned while Bird (1957) had suggested that outer cortex in Ascaris is quinone tanned. There have been suggestions that sulphhydryl groups (which have been shown to be present in this layer) (Carbonell & Apitz, 1961; Anya, 1966a) may participate in quinone tanning (Mason, 1958; Hughes, 1959). Brown (1950) stated that the solution of any structural protein in hypochlorite would indicate quinone tanning, although she admits that the basis of the action of hypochlorite on some structural proteins is not really known.
Polyphenols and phenol oxidases, which are associated with tanning of cuticle, have been demonstrated in the external cortical layer of the cuticles of several nematodes, including *Ascaris* (Brown, 1950; Monne, 1955; Bird, 1957 & 1958a; Watson, 1965b).

The cuticle of *M. incognita* mainly proteinaceous in nature with bound lipids and traces of carbohydrates as shown by various tests in the present studies. Bird (1958b) suggested that cuticle of *M. javanica* and *M. hapla* is a tanned lipoprotein layer which resembles the secreted collagen in their reactions to various reagents. Thus on the basis of Bird's findings and present studies it is suggested that the cuticle of *M. incognita* is mainly lipoproteinaceous in nature with a small amount of carbohydrates (mainly acid mucopolysaccharides) which is further stabilized by tanning, rather than keratin. Tanning is confirmed by the detection of polyphenol oxidase in the cuticle of *Meloidogyne* spp. (Bird, 1958b), besides SH groups of proteins observed in present studies also assist in tanning.

As shown by present observations, the epicuticle of *M. incognita*, too, is a tanned lipoprotein layer. This has also been suggested by Bird (1958b) in *M. javanica* and *M. hapla*. However, besides proteins the cortical layer and basal layer of *M. incognita* show the presence of carbohydrates in traces and a small amount of lipids in the cortical layer. Acid mucopolysaccharides are found in all
the layers of cuticle. Thus the present observations differ from Bird's (1958b) findings who considered the inner layer of *Meloidogyne* cuticle as a homogenous protein layer.

Although Fairbairn (1956) states: "it would seem wise to regard the integument as a fully integrated living tissue rather than as an inert horny sheath", there has been little evidence to show that the cuticle of nematodes is metabolically active.

Any a (1966b) reported RNA in the cortical layers of three parasitic nematodes, *Aspiculuris tetrameres*, *Syphacia obvelata* and *Ascaris lumbricoides*. Chemical analysis of the cuticle of *Ascaris* (Fairbairn, 1957; Bird, 1958a) and histochemical observations of *Aspiculuris* and *Syphacia* (Any a, 1966b) have indicated that a large proportion of the cuticular solids are proteins. Because of RNA in the inner cortex, Any a (1966b) suggests that this layer is also involved in the protein synthesis. In the present studies RNA is being reported in the cuticle of *Meloidogyne* for the first time.

Various tests performed for the localization of non-specific esterases confirm the presence of these in the cuticle (in the cortical layer) of *M. incognita* in the present studies. Lee (1961 & 1962a) observed that the distribution of non-specific esterases coincides with the
distribution of lipids in the homogenous layer of the cuticle of *Ascaris*, as also observed in the present studies in the cortical layer.

Besides, adenosine triphosphatase (ATPase) and acid phosphatase are also reported in the cuticle of *A. incognita* in the present studies. The body wall of filarial worms *Litomosoides carinii* and *Dirofilaria immitis* has been described as strongly positive for acid phosphatase (Maki & Yanagisawa, 1980). Phosphatases have generally been detected histochemically by many workers in sites where absorption, secretion and excretion occur and are postulated to be related with these functions. Ligated worms of *Dirofilaria immitis* have been shown to absorb glucose through body wall (Yanagisawa & Koyama, 1970), confirming the above view. The presence of acid phosphatase in the wall of nematode inhabiting host body fluid provides strong circumstantial evidence in favour of the view that the cuticle of some nematodes particularly those dwelling in the host body fluid, is permeable to water, non-electrolytes and certain ions. In the light of above view same role can be assigned to the presence of acid phosphatase in the cuticle of *A. incognita*, in the present studies. The adult lead an endoparasitic life inside the plant tissue. It may help in the absorption of certain nutrients from the surrounding cell sap of plant tissue. Another probable
function of phosphatase in the cuticle of *M. incognita*
may be that it helps in the absorption of salt to maintain
the osmotic equilibrium.

The presence of acid phosphatase, ATPase and non-
specific esterase in the cuticle of *M. incognita* here,
confirms the findings of Roy (1979). The presence of
these enzymes and RNA in the cuticle of *M. incognita*
suggesting that cuticle, here, too, is in a state of
constant metabolic activity and involved in protein
synthesis. It is also possible that these enzymes are
concerned with the laying down of more cuticle as the
nematode increases in size after final moult.
The hypodermis of *Meloidogyne incognita* is syncytial. In general, the hypodermis lies between the cuticle and somatic muscles. A plasma membrane separating the hypodermis from the cuticle has been reported in several species of nematodes although it may not be of universal occurrence. Chitwood & Chitwood (1950) described the hypodermis of the simplest forms of nematodes of being cellular and this has more recently been substantiated by Maggenti (1961) and Watson (1965a) who found cells in the hypodermis of free-living nematodes. In the more complex forms, however, the hypodermis is usually syncytial, particularly in adult worms.

The hypodermis is very thin in the cephalic region of *M. incognita* females (which grows thicker posteriorly). Similar condition is reported in other nematodes in an area surrounding the stoma.

The hypodermis in the posterior region of the body is projected into four chords.

Nuclei are found more or less evenly distributed in the hypodermis of *M. incognita*, along with a number of
organelles and inclusions. The hypodermal nuclei in the nematodes are usually confined to the chords but the interchordal hypodermis may be nucleate (Chitwood & Chitwood, 1950; Hinz, 1963). The hypodermis particularly in the region of lateral chords of nematodes contains mitochondria and endoplasmic reticulum. Ribosomes (Bird & Rogers, 1965a; Watson, 1965b) and abundant endoplasmic reticulum have been observed in the hypodermis of larvae prior to moulting.

The presence of nerves, nerve cells, glands and duct of the excretory gland in the hypodermis and presence of mitochondria, ribosomes, rough endoplasmic reticulum and Golgi bodies in the hypodermal chords of Meloidosynae is also reported by Bird (1979).

**Histochemistry**

In the present investigations hypodermis of *M. incognita* is found to be rich in glycogen and lipids. Proteins with amino (\(\text{NH}_2\)) and sulphhydryl (\(\text{SH}\)) groups are also reported here.

Little work appears to have been done on the composition of the hypodermis of nematodes, perhaps because it is difficult to separate from the cuticle and muscles. It is well known that fat is present in the hypodermis,
especialiy in the dorsal, ventral and lateral lines of *Ascaris* (Von Kemnitz, 1912; Mueller, 1929; Fairbairn, 1956). Von Kemnitz (1912) regards the hypodermis of *A. lumbricoides* as the principal site of fat storage. Glycogen and fat have been detected in the hypodermal chords of several other genera of nematodes (Fairbairn, 1957; Hinz, 1963; Anya, 1964a). In addition to these, Roggen et al. (1967) have found protein containing vacuoles and leucine-amino peptidase in *Xiphinema index*.

The proteins, RNA and polysaccharides contents of hypodermis of animal parasitic nematodes were also shown by Frandsen (1966), Jenkins (1970) and Singh & Shera (1973) respectively. Johnson (1968) stated the hypodermis to be positive for both proteins and carbohydrates, particularly the latter. In *Enterobius vermicularis* and *Aspiculura tetragona* hypodermis stained intensely by the copper phthaloCyamin method for phospholipids (Anya, 1964a).

The presence of glycogen, lipids, RNA, acid and alkaline phosphatases, glucose-6-phosphatase, succinic dehydrogenase, non-specific esterase and lipase in the hypodermis of *H. incognita* strongly suggests that hypodermis here, as also in other nematodes, is one of the most metabolically active regions. Hypodermis may act as a storage depot for glycogen or it may be involved in some
metabolic process. Presence of enzymes in the hypodermis has been correlated with the synthesis and secretion of cuticular proteins (Lee, 1962a; Januar, 1966). Functions of alkaline phosphatase have been considered by many authors. These considerations involve the processes of secretion, absorption, calcification and formation of fibrous proteins. It seems probable that hypodermis may perform either or all of these functions.

The cuticle of nematodes grows after final moulting (Watson, 1965b) but the synthesis of the proteins of the cuticle has always been assumed to be a function of the hypodermis (Fairbairn, 1960; Watson, 1965b). Such a view finds support in the high concentration of enzymes, reserve food materials such as glycogen, lipids and RNA found in this location in many nematodes (Fairbairn, 1956; Lee, 1960 & 1962a; Anya, 1964a & 1966b; Bemes, 1964; Watson, 1965b) and in the present investigation. The hypodermis in M. incognita is found in close association with the cuticle. The close association of the hypodermis with the cuticle has been regarded as additional evidence in support of the above view.

Studies of the cuticle, however, have failed to reveal substantial quantities of reserve food material in the cuticle, although as already pointed out, the hypodermis shows high concentration of such substances. Moreover,
the hypodermis, by its position and organization, is in intimate contact with the internal tissues and particularly with the body cavity of nematodes. Presumably, the hypodermis serves as a depot from which the material, such as amino acids, needed for the synthesis of the cuticle, are derived. The presence of alkaline phosphatase (which is believed to be involved in the transport of materials in tissues) in the hypodermis of *N. incognita* would support such a view. Alkaline phosphatase has been detected in the hypodermis of other animal parasitic nematodes too (Anyas, 1966a; Watson, 1965b).

Watson (1965b) believes that in *Ascaris* the precursors of the cuticular proteins are secreted from the hypodermis (which she calls epidermis) through a system of canals described by her. This system of canals, however, is not present in all nematodes (Hinz, 1963; Lee, 1965b). The system of canals may be necessary if they were needed for the transport of macromolecules from the hypodermis to the outer layers, of the cuticle. If, on the other hand, the synthesis of macromolecules, which constitute the outer layer, takes place in a location in the outer layers, then only the transport of smaller molecules, such as amino acids or simple sugars, will be involved (Anyas, 1966a).
"There is some disagreement on the manner in which the cuticle is formed in nematodes, particularly with regard to the surface layers. On the one hand (Lee, 1970), it has been reported that a new membrane is formed beneath the hypodermal membrane and cuticular material is then secreted from the hypodermis into the spaces between the two membranes whereas, on the other hand, Borner & Weinstein (1972) have reported that the cuticle is formed externally to the hypodermis plasma membrane. However, it is generally agreed that the cuticle is extrahypodermal in origin" (Bird, 1977).

Bird & Rogers (1965a) in their studies with *M. javanica*, observed under the electron microscope level that at the start of moulting the hypodermis becomes granular and increases in width. These granules resemble ribosomes and are probably associated with the formation of new cuticle. The fibre layer (=basal layer) of cuticle starts to come away from the hypodermis which in turn begins to form an interrupted osmiophilic line, the future external cortical layer of the new cuticle.

The hypodermis probably plays a major role in moulting and also in the rapid growth of the female cuticle after moulting in *Meloidogyne*. The internal mechanisms which are responsible for the stimulation of the hypodermis are unknown.
Wright (1968) reported the presence of mitochondria in the interchordal hypodermis of *Capillaria hepatica* and *Trichuris avocatoris* and suggested active metabolism in this region, while the scarcity of endoplasmic reticulum and Golgi zones implies that protein synthesis and secretion are minimal. The syncytial hypodermis is enriched in DNA has also been reported by Chandler & Read (1961). The rich amount of available carbohydrates (glycogen) in *N. incognita* under discussion may reflect that an additional function of storing the reserve food material is also performed by this layer.

The presence of acid phosphatase in the hypodermis of *N. incognita* confirms the findings of other workers in animal parasitic nematodes (Sood & Kalra, 1977; Maki & Yanagisawa, 1979). Much attention has been paid to the phosphatases in relation to absorption of nutrients. Formerly phosphatases were believed to be involved directly in absorption of nutrients and it has been suggested that their presence may be indicative of active transport (Lumsden, 1975). However, there are many published reports, which contradict the hypothesis that phosphatases are involved directly in absorption of nutrients (Phifer, 1960; Arme & Read, 1960; Dike & Read, 1971; Pappas & Read, 1974;
Levy & Read, 1975; Starling & Fisher, 1975). It has been suggested that the primary function of phosphatases is digestive and they act as intrinsic digestive enzyme and absorption is their secondary function, i.e. products are first hydrolysed then absorbed.
The digestive system in most nematodes is simply a tube into which a number of glands open. The system comprises a stomodaeum (consisting of stoma, oesophagus or pharynx), mesenteron (intestine) and the proctodaeum or hind gut.

*Helodorus incognita*, like other plant parasitic nematodes possesses a stylet, which is moved by protractor muscles. In nematodes the structure, form and mode of operation of oesophagus is very diverse. The lumen of oesophagus is generally triradiate, however, it is not so throughout the organ in all nematodes. In the anterior slender oesophageal part of *Xiphinema index*, lumen is cylindrical (Wright, 1965; Roggen et al., 1967). In *Helodorus* oesophagus is made up of procopus with a cylindrical lumen, which runs from the base of stylet to the median bulb (metacorpus) (Bird, 1979) with a triradiate lumen; the latter is also observed in the present studies.

The oesophagus is thought to be syncytial in all nematodes because cell wall had not been detected by earlier workers (Goldschmidt, 1905; Hsu, 1929 as quoted by Bird, 1971). However, the electron microscope studies
have shown that oesophagus in case of *Ditylenchus dipsaci* (Yuen, 1968a) and *Panagrellus silusiae* (Yuen, 1968b) is a cellular structure with distinct supporting cells, muscle cells, gland cells and nerve cells.

The musculature is usually poorly developed and is mainly confined to the metacorpus (Seshadri, 1964; Yuen, 1968a). In the present studies the metacorpus of *N. incognita* also possesses well developed muscles. In *Meloidogyne* muscle cells have a large number of mitochondria (Bird, 1979).

The intestine of nematodes is a simple tube consisting of a single layer of epithelial cells. In some exceptional cases the lumen of intestine is lost and intestine functions as a food storage organ. This happens in the female of *N. incognita* as in other species of *Meloidogyne*, where there is no connection between the anus and the intestine. In the members of Mermithidae the connection with both pharynx and anus is lost. In *Meloidogyne* intestine retains somewhat cellular structure with cell borders visible in TEM but the characteristic intestinal lumen with microvilli is missing (Dropkin & Aceto, 1974). The intestine appears as an opaque mass of large fat globules and clear evidence of cell walls in adults of *Ditylenchus dipsaci* and *Aphelenchoides paratitus* is lacking (Chitwood & Chitwood, 1950).

In *N. incognita*, in the present studies, the nuclei are found to be scattered throughout the stored food granules
in the intestinal mass. Similar observations were made in *M. hapla* (Elsea, 1951) and *M. incognita acrita* (Maggenti & Allen, 1960).

In *M. incognita*, there are three uninucleate oesophageal glands, one dorsal and two subventrals. The two subventrals are highly reduced in adult females. The oesophageal glands may be multinucleate or uninucleate and are three in number as in Spirurida, Enoplida and Dorylaimida, in *Longidorus*, they are 5 (Aboul-Eid, 1969).

The structure of oesophageal glands of *M. javanica* has been studied by Bird (1967, 1968a & b, 1969) and Bird & Saurer (1967). In adult females of *Meloidogyne* the dorsal oesophageal gland is enlarged, as also observed in the present studies. This gland has an important role in feeding, first demonstrated in the genus by Linford (1937) and subsequently demonstrated in various other nematodes.

In the present studies, females of *M. incognita* possess six large unicellular rectal glands around the rectum. These glands open through a common duct into the rectum. They produce gelatinous matrix which surrounds the eggs. In nematodes, depending on the sex, the number of rectal glands varies in different species and also varies within the same species. A detailed study of these rectal glands has been carried out by Maggenti & Allen (1960), Minton (1965) and Bird & Rogers (1965b).
Intestine of adult female *M. incognita* loses all cellular identity and is a syncytium. Intestinal nuclei can be seen scattered along with the stored food granules. Presence of protein granules (NH$_2$ and SH groups), amino acids, lipid droplets (both phospholipids and neutral lipids), carbohydrates (glycogen, PAS positive granules), RNA and lipoprotein bodies indicates that intestinal mass acts as a storage depot for reserve food material. Acid mucopolysaccharides and metachromasia are also reported in the intestinal mass.

The presence of various macromolecules (proteins, lipids and carbohydrates) in the intestine, hypodermis and cuticle suggest that these are transported from the intestine to the cuticle through hypodermis, to the hypodermis itself and also to developing ova.

Intestinal mass because of the presence of various macromolecules can be compared with the perienteric fluid of Anya (1976) from where essential metabolites may be transported to the developing oocytes through the epithelial cells of reproductive organs. The transport of material is further supported by the presence of acid phosphatase and phospholipids in the intestinal mass, which may assist
In Thelastoma bulhoeai the intestine is an important organ of food reserve. Lee (1960) observed that in the adult female of Thelastoma bulhoeai the cells of the middle intestine are rich in glycogen and fat. In the intestine of Cephalobellus papilliger and Blatticola blattae fat is the chief form of stored food (Chitwood & Chitwood, 1950). Von Kemnitz (1912) and Hirsch & Bretschneider (1937) found large quantities of glycogen and a small amount of fats in the intestine of Ascaris lumbricoides and A. eequorum. However, Toryu (1933), found only a small amount of glycogen in the intestine of A. eequorum. Giovannola (1936) found small globules of fats in the lumen of free-living stages of certain parasitic nematodes.

It is possible that the fat droplets in the cells of the intestine have been shed into the lumen to be broken down by the lipase of the gut into easily absorbed and transported food (Lee, 1958).

Hobson (1948), after reviewing the results obtained by various workers, stated that fat present in parasitic nematode is true reserve food which is not normally used as a source of energy by the nematode because of the low
oxygen tension usually present in the intestine, and because large amounts of glycogen are more readily available. He also stated that if sufficient oxygen is available, at certain stages of the life history, the fat is almost certainly broken down.

RNA is also found in the intestinal mass of *M. incognita*. Presence of RNA in the intestinal cells of *Ascaridia galli* corresponding to ultrastructural ribosomes and granular endoplasmic reticulum in other species (Colan, 1970) can be correlated with protein synthesis.

Glucose-6-phosphatase enzyme is present in the form of granules throughout the intestinal mass scattered in between the intestinal droplets. Glucose-6-phosphatase presumably has a role in digestion and absorption of nutrients as stated by Lumsden (1975) and Pappas & Read (1974) in case of helminths.

In the present studies it has been found that non-specific esterase is present in the form of small and large granules in the intestinal mass. "Caps" of enzyme also seem to be present on the fat droplets. Besides, lipase is also found associated with non-specific esterase. Lipases or esterases are detected in the extracts of intestine of various nematodes and of the whole nematode by Flury (1912), Rogers (1941), Carpenter (1952).
Lee (1958) and Nimmo smith & Keeling (1960), though their investigations have not revealed whether the enzymes are secreted into the lumen of the intestine for extracellular digestion or are intracellular enzymes. The maximum enzyme activity occurred in the foregut which also contains the secretory granules (Carpenter, 1952). In *A. lumbricoides* esterase present in the contents of lumen, show clearly that *Ascaris* carries out extracellular digestion and that secretion of the enzyme occurs along the length of the intestine but especially in the anterior part of the intestine (Lee, 1962a). Non-specific esterase present in the oesophagus of *N. incognita* in the present studies may help in extracorporeal digestion of the cell contents it feeds upon and in the intestinal mass esterase and lipase may help in extracellular digestion and lipase may assist in preparing lipids for absorption from the intestinal mass.

Metacorpus of female *N. incognita* shows the presence of enzymes cytochrome oxidase (CO), succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase). The muscle cells of metacorpus of female *H. maloidogyna* are well supplied with mitochondria (Bird, 1979). This fact itself supports the presence of above enzymes in the metacorpus and suggests that these enzymes are involved in the process of energy production which may be required for the movement
of muscles at the time of feeding.

The dorsal oesophageal gland and its content in the dorsal oesophageal gland and its content in *M. incognita* mainly consist of proteins and carbohydrates but do not show the presence of nucleic acids, lipids, acid phosphatase, alkaline phosphatase, CO and SDH. This is in conformity with the findings of Bird & Saurer (1967); Bird (1968b) in *M. javanica*. They observed these components to be present in the form of granules. These granules may contain enzyme precursors which lead to the production of enzymes when in contact with the cytoplasm of the host (Bird, 1971). In *M. javanica* the substances secreted by the dorsal oesophageal gland contain basic proteins (with some properties of histones) as well as glycoproteins and mucopolysaccharides (Bird, 1968b) which are responsible for the development, maintenance and control of the tumor like giant cell in plants on which these nematodes feed (Bird, 1961).

Rectal glands in *M. incognita* in the present studies show the presence of PAS positive material, acid mucopolysaccharides, metachromasia, RNA and traces of acid and alkaline phosphatase confirming the findings of Bird & Rogers (1965b) in *M. javanica*. PAS positive material in *M. incognita* is present mainly in the multivesicular lamellar bodies. Bird & Rogers (1965b) observed Golgi bodies in the rectal glands of *M. javanica*. According to
then the PAS positive material in the Golgi region consists of glycoprotein and/or mucopolysaccharides.

According to Kurosuni (1961) mucus droplets are formed from Golgi bodies. The close relationship of Golgi body and multivesicular lamellar body may be responsible for the production of mucopolysaccharide (Bird & Rogers, 1965b). The multivesicular lamellar body resembles the multilayered structures which have been described for a range of phospholipids systems (Finean, 1961; Buvat, 1963). In _M. incognita_ rectal glands secrete gelatinous matrix. Gelatinous matrix in _Maloidocryne_ is a tanned protein containing acid mucopolysaccharides and a number of enzymes (Bird & Rogers, 1965b).
FEMALE REPRODUCTIVE SYSTEM

HISTOLOGY

The female reproductive system of most nematodes consists of two paired genital tubes which show regional differentiation (Bastain, 1866; Seurat, 1920) as also found in case of Helodogynae incognita in the present studies. In telogenic forms germinal area at the blind end of the ovary is short (Van Beneden, 1883). The ovary of H. incognita is telogenic i.e. it consists of a short germinal zone followed by a more or less elongated growth zone.

The overall morphology is like that of related members of the Tylenchidae and other nematodes (Geraert, 1972). Oocytes of H. incognita originate in the germinal zone of the ovary. In the germinal zone oogonia are arranged around a central anucleate rachis. In H. javanica oogonia are attached by cytoplasmic connexions to the central rachis (Molure & Bird, 1976). The rachis consists of non-nucleate tissue containing lamellar bodies, dense granules and what appear to be ribosomes (Foor, 1967 & 1968).

In Heterakis gallinarum the rachis not only serves as a support for developing oocytes, but also makes possible synchrony of germ cell development (Lee & Lestan, 1971). The rachis of H. incognita seems to perform the same function.
As the oocytes mature and increase in size, they become detached from the rachis. Mature oocytes passing through the oviduct enter a region of the gonad which has been termed variously a spermatotheca-oviduct valve, a sphincter, or a constriction (Geraert, 1972). Triantophyllou (1962) considered this structure in *M. javanica* to be the oviduct itself. Ultrastructural studies of the cells of this region in *M. javanica* suggest that they are sites of great metabolic activity (Mulure & Bird, 1976).

The spermatotheca apparently is a constant feature within the Tylenchida. However, its morphological and histological details show considerable variations (Geraert, 1972; Yuen, 1964). The spermatotheca usually unites in a straight line with the uterus as in case of *M. incognita* but may join the latter in a well pronounced angle (*Heteroderidae*) or it may set off dorsally as in *Helicotylenchus* or lateroventrally as in *Pratylenchus*. In *Helicotylenchus* and *Pratylenchus* another constriction may occur between oviduct and spermatotheca (Hirschmann & Triantophyllou, 1967; Roman & Hirschmann, 1969).

The spermatotheca opens into the uterus, which is a broad tube. The uterine musculature is generally poorly developed in plant parasitic Adenophorea and Secernentea. In *Xiphinema* a sphincter muscle is present between the oviduct and uterus (Coomans, 1964) and mid uterus is provided with
muscles (Hirschmann, 1971). The uterus here in case of *N. incognita* can be divided into distal, mid and proximal portions. The cells of the uterine wall in the distal portion contain large number of intra cytoplasmic spaces. The cytoplasm of the cells in the mid region is dense and much more compact in the proximal portion with very few intracytoplasmic spaces as also reported in *N. javanica* (McLure & Bird, 1976). In *X. chinensis* the distal portion of uterus may function as a spermatheca and may play a role in the formation of egg envelope. Apart from the investing muscle cells, uterus in nematodes consists of squamous epithelial cells (Anya, 1964b; Hope, 1974).

In *N. incognita* the proximal portions of the uteri enter a common tube, the vagina. The lumen of vagina is lined with cuticle which is in continuation of outer cuticle, and opens to the exterior through vulva. Generally vulval opening in nematodes lies in the middle of the body but in some forms it may open anteriorly or posteriorly (Bird, 1971). Vulva in *N. incognita* is located terminally as in case of *Heterodera*. In some plant parasitic nematodes, viz. *Pratylenchus* and *Hoplolaimus*, vulval membranes also occur (Hirschmann, 1971).

In the present studies, muscles of vagina of *N. incognita* are well developed. The lumen of vagina is
narrow, which gets dilated at the time of egg laying
with the help of vaginal muscles. In parasitic nematodes,
the vagina is greatly elongated and muscularized for the
increased production of eggs and their ejection. In some
nematodes it is called ovjector (Seurat, 1920). In
*M. incognita* the egg shell formation is initiated in the
distal part of the uterus. The egg shell formation in
*Acaria lumbricoides* occurs in uterus and is stimulated
by fertilization and perhaps dependent upon it (Poot, 1967).
In *Meloidogyne* reproduction proceeds through mitotic
parthenogenesis in the absence of male (Triantaphyllou,
1962).

**Histochemistry**

In the present studies epithelial cells of germinal
zone are found to be rich in glycogen. While the oogonia
are devoid of it suggesting that glycogen has not yet
appeared in these cells. In the growth zone amount of
glycogen decreases in the epithelial cells whereas it
increases in the oogonia. The amount of glycogen in
epithelial cells adjacent to the mature oocytes is much
less as compared to those adjacent to the maturing oocytes
and oogonia thus suggesting a transfer of glycogen from
epithelial cells to the developing oocytes.

In the upper region of the growth zone the epithelial cells of the ovary of *Ascaris* contain "secretory vesicle" having glycogen (Prestage, 1960). Besides glycogen, a large number of mitochondria and endoplasmic reticulum have also been reported in the cytoplasm of these cells (Prestage, 1960).

Proteins and lipids are also being reported in the epithelial cells of reproductive system of *Maloideozymes incognito*. Comparatively more proteins and lipids are found in the walls of oviduct and uterus than in the walls of germinal zone and growth zone. Cytoplasm of these cells reveals the presence of glycogen, proteins with NH₂ and SH groups, lipoproteins and phosphatase. In *M. incognito* the germ cells are comparatively more pyroninophilic as compared to the epithelial cells in their immediate vicinity. The structure of epithelial cells in nematodes suggests that these themselves are non-secretory and simply help in the diffusion of nutritive material from the surrounding environment into the ovary. Since the epithelial cells of ovary in *M. incognito* are rich in proteins, carbohydrates and lipids, it is conjectured that they provide nourishment to the developing ova. Epithelial cells of uterus may have an additional role in the formation of egg envelopes.

Raven (1961) suggests that auxiliary cells in vertebrates play an important role in the nutrition of
maturing oocytes, presumably taking up substances of low molecular weight from their environment, synthesizing them into higher compounds, which are then transmitted to the egg cells. In the light of these observations of Raven (1961) it is possible that the epithelial cells in *Meleagrogyne*, too, have similar function to perform, i.e. these cells absorb nutritive material from the intestinal mass to be passed on to the developing oogonia and oocytes. As Chitwood & Chitwood (1950) and Anya (1976) have stated the ovary with its epithelial monolayer of cells, is suspended in the body fluid, the perienteric fluid, which is a complex mixture of proteins, amino acids, carbohydrates, trehalose, glucose, fats and inorganic ions (Hobson, 1948; Hobson et al., 1952a,b; Pollak & Fairbairn, 1955; Fairbairn, 1960). The perienteric fluid would thus provide a rich pool of metabolites for the active synthesis of proteins necessary for the full development of developing oocytes. The investing epithelial cell layer does exert a selective effect on the entry of amino acids into the ovarian tissue (Viglierchio & Gortz, 1972). However, the ultrastructural features of the epithelial cell do not indicate an active synthetic role (Foer, 1967; Lee & Lestan, 1971). Kochhar (1960) maintains that "follicular epithelium contributes lipoprotein bodies and glycogen to the oocytes. The lipoprotein bodies are essential for the production of the
hyaline spheres (protein yolk). This suggests that epithelial cells are also involved in some part of vitellogenesis besides perienteric fluid, which is a rich source of various metabolites.

Poor (1968) suggests that smaller protein molecules could be synthesized in the alimentary cells, secreted into the perienteric fluid and subsequently abstracted therefrom by the epithelial cells. The role of the intestinal mass of *Meloidogyne* is comparable to that of perienteric fluid of other nematodes.

Acid mucopolysaccharides along with metachromasia are also found to be present in the epithelial lining of ovary of *M. incognita*. Monne (1959) and Katchalsky (1964) suggest that acid mucopolysaccharides, in general, are associated with physiologically active absorptive surface and help in biological cation exchange. Thus acid mucopolysaccharides in the epithelial cells in *M. incognita* may also assist in increased permeability and in the transport of material through these cells to the developing oocytes. Transport of metabolites through epithelial cells is further confirmed by the presence of alkaline phosphatase in these cells, as phosphatase also helps in absorption, secretion and excretion (Maki & Yanagisawa, 1980). The
above views are in conformity with those of Anya (1976) who reported that the nutrients are transported from the perienteric fluid to the oogonia through these cells. Esterase and lipase enzymes are found in the epithelial cells of the ovary of *M. incognita*. In the present studies the distribution of lipids together with esterases in the reproductive system of *M. incognita* suggests that the system is a site for extensive metabolism of lipid in conjunction with the production of eggs. Enzyme is mainly present in the epithelial cells which is probably used in the supply of nutrients to the developing germ cells. The presence of esterase in the oviduct and uterus is probably concerned with egg shell formation (Lee, 1962a).

**Rachis** is a characteristic feature of the ovary of telenogonic nematodes (Chitwood & Chitwood, 1950). The structure is annulate and its cytoplasm contains glycogen, lipid droplets, microtubules, dense inclusions and ribosomes (Prestage, 1960; Poor, 1968; Lee & Lestan, 1971; McLaren, 1973). Varied functions have been assigned to the rachis. Bartschlii (1873) and Mayer (1908) compared the rachis of *Ascaris* with the verson's cell of Lepidoptera. In the ovary of nematodes there is no equivalent of nurse cells of insects and other invertebrates or of follicle cells of vertebrate ovarian tissue. Mayer (1908) suggested that rachis may have a
nutritive function in nematode ovary. The absence of a well defined nucleus in the rachis suggests a limited ability to synthesize ovarian yolk and their precursors (Foor, 1968). Two alternative functions of rachis are suggested by Prestage (1960). Either it serves as a reserve store of excess materials, synthesized originally by the oocytes and sequestered by the rachis until needed; or alternatively, it serves as an absorptive and synthetic "tank" from which nutrients may be drawn by the oocytes as and when required. The second view is further supported by Anya (1976) who observed the presence of intercellular microtubules connecting the developing oocytes and the rachis as well as certain bodies of an apparently protein nature. Foor (1968) has ascribed the synchronization of oocyte development to the rachis. However, Anya (1976) suggests that nutrition of oogonia and oocyte and its associated vitellogenesis depends upon the abstraction of nutrients from the parienteric fluid, followed by synthesis in the epithelial cell and the oocyte itself rather than in rachis.

In the rachis of *M. incognita* esterase is also found to be present, which may help in lipid metabolism.

During the present studies a gradual increase in the cytoplasmic inclusions is observed in the rachis and developing oocytes so it is reasonable to suggest that
the oocytes themselves incorporate and assimilate nutritive materials and the inclusions within the rachis are observed only after their initial appearance in the oocytes. In *Ascaris*, Von Kermann (1912) observed that glycogen appeared in the rachis only after this substrate had become the chief stored product of the ovary.

In the present studies a gradual increase of glycogen, proteins and lipids is observed from oogonia to eggs in *N. incognito*. The first cytoplasmic inclusions which appear in the early gonial cells are lipid droplets (Flury, 1912; Faure-Fremiet, 1913; Lee, 1960; Monne, 1959; Anya, 1964a; Foor, 1967). Then large amount of glycogen and ascaroside esters appear in the developing oocyte (Faure-Fremiet, 1913; Fairbairn, 1957). Large quantities of glycogen are observed in the oocytes of all the nematodes studied (Flury, 1912; Faure-Fremiet, 1913; Kocshar, 1960; Lee, 1960; Anya, 1964a).

The reproductive organs are the most important site for lipid deposition in female *Ascaris* and perhaps in other female nematodes (Fairbairn, 1960; Lee, 1960 & 1962a; Anya, 1964a). However, Von Kermann (1912) was unable to detect fats in the ovary of *Ascaris*. Lipids in reproductive system are mainly neutral (Faure-Fremiet, 1913) and in developing embryos they serve as substrate for carbohydrate
synthesis (Fairbairn, 1955b). In many nematodes it is now generally accepted that fatty acids are utilized in glyco-
genesis (Pasey & Fairbairn, 1957; Baret et al., 1970).

In the cytoplasm of the oocytes of *Parascaris equorum* three types of inclusions, viz. homogeneous droplets, hyaline granules and refringent granules are described by Van Beneden (1883). The presence of hyaline granules and refringent granules is further reported by various workers in different nematodes, in *Acaria lumbricoidea* (Yanagisawa, 1955; Foor, 1967), in *Porrocaecum aquatilicola* (Kochhar, 1960), in *Ampiculuria tetrantera* (Any, 1964b) and in *Heterakis gallinarum* (Lee & Lestan, 1971). These inclusions are supposed to be present in all those nematodes where egg shell formation is an essential part of fertilization process (Any, 1976).

In the present studies, a large number of lipoprotein granules, refringent protein bodies, PAS positive granules are also observed in the developing oocytes and eggs of *H. incognita*.

In the mature oocytes of nematodes, yolk includes lipid droplets, glycogen reserves and the dense granules. The glycogen, lipid droplets and the ascaroside esters of the refringent granules are elaborated within the developing oocyte (Fairbairn, 1957; Lee, 1963; Any, 1964b; Jesyk &
Fairbairn, 1967). The dense granules are synthesized extracellularly. These granules are deposited on the plasma membrane and oocytes takes it through the process of micropinocytosis (Foer, 1967).

In the oocytes of P. angusticolla, Kochhar, (1960) described two types of yolk, viz. the lipid yolk and the protein yolk (also called hyaline spheres). He suggested that lipid yolk consists of triglycerides and is formed by the transformation of phospholipids bodies of the early oocyte. The hyaline sphere on the other hand, consists of ribonucleic acids derived from the nucleus and the proteins and carbohydrates from the cytoplasm. These are synthesized in close association with the lipoprotein granules contributed to the oocyte by the follicular epithelium.

In A. tetraptera, Anya (1964b) states that the cytoplasm of the mature oocyte, in addition to large refringent spheres and hyaline granules. The hyaline granules contain predominantly proteins with which phospholipids may also be associated, whereas refringent bodies contain very little, if any protein. Fouquey et al. (1957) have shown that in Ascaris, these refringent spheres consist of glycosides which have so far been found only in nematodes. Molure & Bird (1976) have observed a large number of lipid
droplets, glycogen and numerous refringent protein bodies in oocytes of *H. javanica* as also found in *M. incognita* in the present studies.

The oocytes of *Heterakis gallinarum* contain two types of granules, the refringent granules which give rise to the ascaroside layer of the egg shell and another kind (type 2 granules) which appear to be a kind of yolk for the developing egg (Lee & Lestan, 1971). In the oocyte of *Enterobius vermicularis*, Kulinska & Kulinsky (1973) discovered two types of granules i.e., small darkly staining lipoprotein granules and somewhat large protein granules.

It is presumed during the course of present studies on *M. incognita* that the lipid droplets and PAS positive granules provide the nutritive material whereas the cytoplasmic glycogen and the lipoprotein granules seem to contribute in the formation of the egg shell.

As far as the nematodes are concerned, most of the workers have not reported any Golgi material in the female germ cell. Lee & Lestan (1971), however, have described in the early oocytes of *Heterakis*, a few Golgi bodies which reportedly decrease in number as these oocyte mature. Further Lee & Lestan (1971) conjecture that the decrease in number of Golgi element is related with
the formation of refringent granules and the yolk granules. The lipoprotein granules observed in the oocytes of *M. incognita* are comparable with the Golgi bodies of Lee & Lestan (1971).

In *Ascaris lumbricoides* and *Aspiculirus tetragaster* the protein moiety of the chitinous layer is derived from the pre-existent and proteinaceous hyaline granules of the oocyte as suggested by Yanagisawa (1935) and Anya (1964b). However, according to Poor (1967) and Lee & Lestan (1971) hyaline granules do not contribute any material in the formation of egg shell in *A. lumbricoides* and *Heterakis gallinarum*.

Kuchenmeister (1857) and Christenson (1950) suggested that the secretion of the cells of upper region forms the lipoprotein layer of the egg. This view is supported by Anya (1964b) and Poor (1967). They found that uterine cells secrete strands of lipoprotein material which becomes deposited on the egg surface to form the outermost lipid layer.

Alkaline phosphatase activity was observed in the region of the forming chitinous layer of *Aspiculirus tetragaster* (Anya, 1964b), but was not detected in *Heterakis gallinarum* (Lee & Lestan, 1971). The presence of acid phosphatase in the oocytes of *M. incognita* suggests that it may help in partial digestion of cell metabolites.
The presence of adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH) and cytochrome oxidase (CO) enzymes in the eggs and in the muscles of vagina suggests that these structures are involved in various energy yielding processes. Since vagina is actively involved at the time of egg laying requires a lot of energy for muscular contraction further supports the presence of these enzymes in this structure. Non-specific esterase and lipase found in these structures may assist in lipid metabolism. Lipases, presumably assist in lipid movements within the tissue rather than preparing lipids for absorption from the intestinal tract (von Brand, 1966).
MODE OF ACTION OF NEMACUR ON *HELIOCOCCUS INCognita*

The review of literature reveals that organophosphate nematocides have been shown to act through inhibition of esterases in general and cholinesterase and acetylcholinesterase specifically. However, the way they affect the general physiology of nematode is not readily understood.

Nematocides like other drugs, must reach the site of action in the parasite before they can exert their effects. The toxic molecule reaching inside the nematode, may become stored in non-target tissue; alternatively it may be excreted, detoxified or converted from a less toxic precursor to a toxicant. For the action of nematocides four possible sites are proposed (i) neuromuscular co-ordination (ii) energy metabolism (iii) haemproteins and (iv) membrane lipoproteins.


In the present studies, too, *H. Incognita* is found to inhibit the esterases. According to del Castillo (1969) acetylcholine causes contraction of muscles of *Ascaris* and the extent of contraction is directly related to the
concentration of acetylcholine used. Morban & Vigliarchio (1980) suggest that in *Pratylenchus vulnus* organophosphate and carbamate act by interfering with the transmission of nerve impulses to somatic musculature. The inactivity of the larvae of *M. incognita* in the present studies, after treatment with Nemacur can also be attributed to the inhibition of muscular esterases. In case of female inactivity, however, it cannot be directly related to the inhibition of muscular cholinesterase, because muscles are not well developed here.

Present studies reveal that after treatment with Nemacur cytochrome oxidase (CO) is totally inhibited in case of female *M. incognita*. Dietze & Kemple (1973) have found that carbamate and organophosphate pesticides inhibit CO of *Panagrellus redivivus* and *Rhabiitis oxyerca*. The activities of succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase) are also significantly reduced. Since CO is the terminal member of the electron transport chain involved in energy metabolism (mitochondrial oxidative phosphorylation) its inhibition would lead to the inhibition of ATP synthesis resulting in the decrease level of ATP in the system. The inhibition of ATPase would further cause disruption of energy metabolism.

The overall decrease of ATP production would be expected to cause a decrease in the rate of anabolic processes resulting in the lowering of the concentration of various biomolecules,
proteins and acid mucopolysaccharides are reduced after treatment with Nemacur. Ishibashi (1970) observed certain changes after treatment with EDB (an alkylhalide nematicide) which are suggestive of protein denaturation.

In general the decrease in ATP should affect the synthesis of all the amino acids uniformly. The relatively great decrease in the SH containing amino acids observed in the present studies could be attributed to the fact that SH groups of the amino acids may not be free and present in the form of a complex with Nemacur. Thus the greater decrease in the level of these amino acids observed in the present studies could be due to the limitation of the method of detection rather than the actual greater decrease in the level of these amino acids.

According to Moje (1960) sulphydryl group blockage may be most important reaction in alkyl halide nematicide toxicity. Evans (1973) suggests that EDB (an alkyl halide nematicide) inhibits enzyme reaction by blocking potential sites for nucleophilic substitution reaction (sulphydryl groups, amino and hydroxyl groups).
The inhibition of cytochrome oxidase, ATPase and SDH would not only lead to the decreased synthesis of ATP but also to the accumulation of reduced potentials in the form of NADH, NADPH and FADH$_2$. The increase in their concentration may lead to the inhibition of catabolic reactions which are primarily oxidative in nature and which require the oxidized form of the above cofactors.

ATPase activity is known to be related to energy metabolism, active electron transport and lipid synthesis (Lehniger, 1975). Change in ATPase may be correlated to above mentioned functions.

During nematocidal intoxication, alternate pathways for energy metabolism also appear to be activated (Evans, 1973). To meet the energy requirements of the system the inhibition of oxidative phosphorylation directly and the decreased level of catabolic reactions indirectly would lead to the greater mobilization of building blocks from body stores e.g. glycogen. In the present studies upon treatment with Nemacur increase in G-6-Pase activity was observed. This could either mean increased mobilization of glucose from glycogen and/or increased rate of gluconeogenesis to produce glucose to meet its requirements for certain vital functions of the body. Body regulatory mechanism would be expected to act in a way to maintain the energy requirements of the body. In order to do so they will have to degrade
more of glucose under the condition when ATP generation by oxidative phosphorylation is completely inhibited.

Kampfe & Ritzrow (1970) have observed that the respiratory metabolism of *Panagrellus redivivus* is affected when the nematode is treated with carbamates and organophosphates. They have observed the effect of lethal concentrations of nematicides which is characterized by the death of the nematodes. They have examined the respiratory metabolism of *Rhoditis oxyerca*, *Panagrellus redivivus* and *Heterodera schachtii* by means of manometric methods. For this purpose effective substances (e.g. Dazomet, Temik and an organophosphorus compound) were used in low concentrations. Generally the lowest concentration has been found cause an increase of the $O_2$ consumption expressed in ml/mg dry weight of the nematodes. Higher doses diminish the respiratory activity considerably. Each agent does influence the animals in a different degree. There were no sign of recovery during the experiment.

A decrease in lipoproteins observed in the present studies is attributed to the general decrease of anabolism resulting from the decrease in ATP production. Since cuticle of *Meloidogyne* is mainly lipoproteinaceous in nature which by forming a protective covering is responsible for the selective permeability of the membrane, it may be possible that a decrease in lipoprotein component may provide
a rapid entrance of nematicide through the cuticle to kill the nematode. A decrease in the acid mucopolysaccharides observed in the present studies would further lead to the disruption of the selective permeability of the membrane.

Albert (1968) has suggested that the receptors are the sites within lipoprotein membranes where the nematicide binds. This supports the above assumption in case of *M. incognita*. Cholinesterase helps in nerve transmission in general. Besides there are some other probable functions, which can be assigned to the esterases present in the amphidial glands of female.

In the amphidial pouches of adult female *M. incognita* cholinesterase is found to be present associated with non-specific esterase. Enzyme activity gets inhibited after treatment with Nemacur.

It is possible that the secretion of amphidial glands (which may be under the control of cholinesterase present in the gland) is responsible for the movement of muscles holding the stylet and ultimately with feeding. The secretion of gland may also be associated with some vital functions of *M. incognita*. Thus the inhibition of enzyme after treatment with Nemacur may check the vital functions of the female like feeding etc., which may be the cause of its death. A possible function suggested by Lee (1969) and
Ogilvy & Jones (1971) is that the enzyme might function as a biological "hold fast" allowing nematodes to maintain their position amongst the intestinal microvilli. In view of this it can be conjectured that enzyme here may help in maintaining the position of female within the giant cell, thus again responsible for feeding.

Lee (1970) has suggested that the enzyme might alter the permeability of the host membranes and thus facilitate the leakage of nutrients. This can be true in case of *M. incognita* too, where enzyme may help in altering the permeability of membranes of giant cells and various organelles which are present in the giant cells, from where food comes for females.

According to Geraert (1965) sexual attraction function can also be assigned to the amphidial secretions.

Thus, the esterases present in the amphidial gland inhibited once after treatment with Nemacur will check all the possible functions assigned above which results in the death of females of *M. incognita*.

The mode of action of Nemacur on *M. incognita* has been summarized in the following page.
1, 2, & 3 are the receptor sites. 2 is the main receptor site.

**Hypothesis for the Mode of Action of Nemacur**

1. **NEUROMUSCULAR CO-ORDINATION AND VITAL FUNCTIONS**
   - Inhibits esterases (ChE + NSE)
   - SH group blockage
   - disturb

2. **ENERGY METABOLISM**
   - Reduction in ATP production
   - Accumulation of reduced potentials viz. NADH, NADPH & FADH$_2$
   - Decrease in the rate of anabolic processes
   - Inhibition of catabolic reactions
   - Decreased level of various biomolecules viz. lipids, proteins, carbohydrates &

3. **LIPOPROTEINS**