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Full Length Research Paper

Histological study on the body wall of *Ascaridia galli* (Nematoda)

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The body wall of Ascaridia galli has been investigated using 1-2 µ thick sections and stained with Toludin Blue. It is found to consist of three layers, cuticle, hypodermis or subcuticle and muscle layer. The cuticle in turn consists of three layers namely cortex or upper layer deeply stained with TB, 1.7 µ in thickness, middle layer or matrix 3.15 µ in thickness with no distinct constitutions, then fiber layer which in turn consists of several layers of dense tissue, its thickness 5.65 µ and settled on basement membrane. On the surface of the cuticle plugs a transverse annuli which divide the body into annuli, the distance between each two 0.22 µ each annulus is divided by three subannuli, large anterior 0.124 µ in length and other two are small, middle and posterior 0.047-0.054, 0.045-0.054 µ respectively. The hypodermis or dermis is thin and not well developed and of syncytial type and its nuclei are constricted to the longitudinal cords that is, dorsal, ventral, and two laterals. Mainly these nuclei are present in the lateral cords and longitudinal excretory canals which pass through these cords. The hypodermis become more thicker as proceed posteriorly to reach 2 - 3 µ in thickness in the inter-caudal region as well as thicker in the four longitudinal cords protruded in the pseudocoel and reach the maximum thickness near the nerve ring. In addition to these cords there also four secondary cords which divide the muscle groups into 8 sectors and. Moreover lateral cells in the middle of the body are larger and more prominent becoming pyriform clusters or cup-shaped forming lateral hypodermal glands. The average depths of these glands are 97 µ in male and 148 µ in female. A canal or duct arises from each gland the two canals united to open together and a prominent cuticular pominance is seen near the opening of these glands.

Key words: *Ascaridia galli,* cuticle, nematode, layer, cortex.

INTRODUCTION

The body wall of nematodes has been subjected to investigation especially those of Ascaris lumbricoides and other ascarids (Bird, 1971; Chitwood and Chitwood, 1974), as they studied cuticle and its layers and its chemical compositions. Roggen et al. (1967) studied the body wall, cuticle, hypodermis, and muscle layer of *Xiphinema ibndex* which is a plant parasitic nematode .A detailed ultrastructural study has been performed on the body wall of potato parasitic nematode, Heterodera rostochiensis (Wisse and Daem, 1968). In addition the body wall of the free living nematodes, *X. xenoplax* has been investigated especially its cuticle layers, hypodermis, and body muscles (De Grisse, 1972).

Ultrastructure of *Syphacia obvelata*, a roden nematode, observed the cuticular modification especially the middle layer in the lips, papillae, buccal cavity by Wright and Hope (Wright and Hope, 1968), while Batson (1979) demonstrated the ultrastructure of *Gastromermis boophthorae* and its larvae and its relation with nutrition. Lee et al. (1993) made a freeze-fracture study on the cuticle of adult *Nippostrongylus brasiliensis*. Nawab-Al-Deen (1994) studied the cuticle ultrastucture of the fish parasitic nematode, *Rhabdochona tigrae*. DeCraemer et al. (1996) studied the cuticle ultrastructure of the free living nematodes, *Criconema paradoxiger* especially the external annulation.

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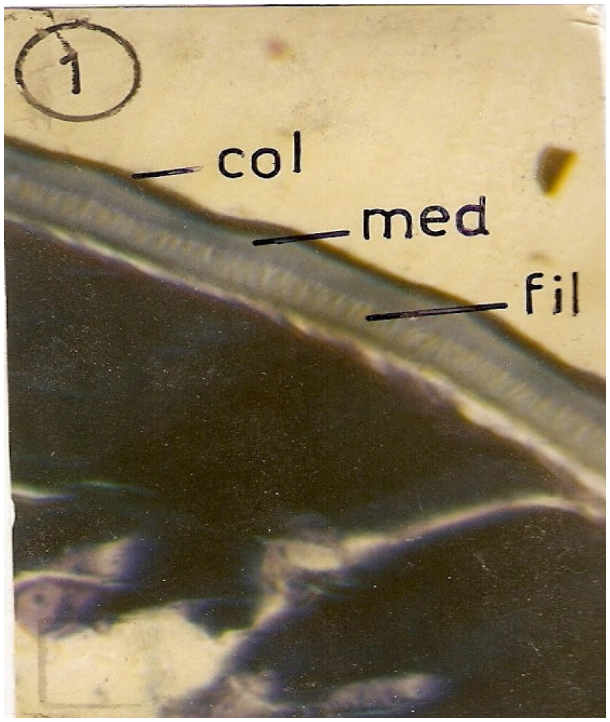

Figure 1. Photomicrograph of A section passing through the cuticle layers of *Ascaridia galii*.
Col; cortical layer, **med;** median layer, **fil;** fibrous layer. Toludin Blue X400.

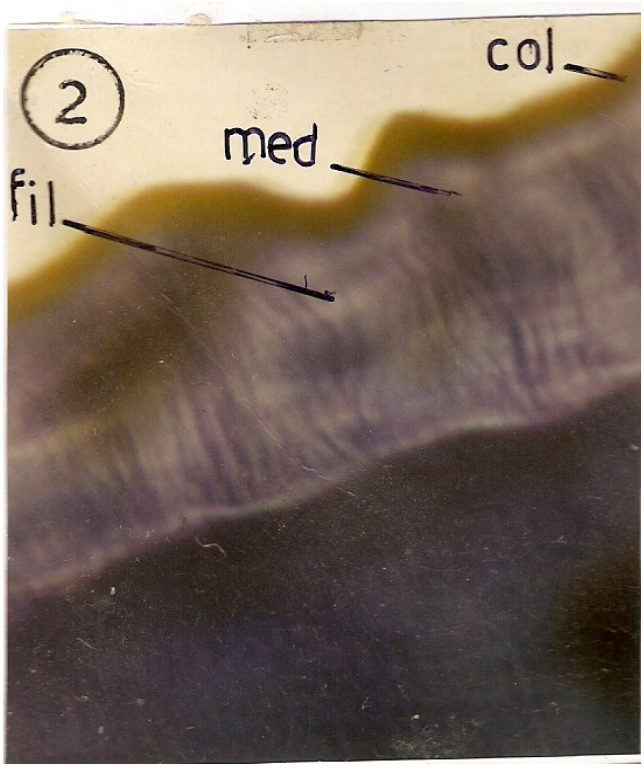

Figure 2. A magnified photomicrograph of a section passing in the body wall of cuticular layers
Col; cortical layer**, med;** median layer, **fil;** fibrous layer. TB ×1000.

MATERIALS AND METHODS

Worms of A. galli recovered from local fowl, Gallus gallus domesticus which have been bought from local markets in Mosul. Worms were removed, washed in Hanks solution, pieces were fixed in gluteraldehyde dissolved in 2% phosphate buffer for 60-90 min in ice bath (4 C), and then specimens were moved to 1% osmium tetra oxide in phosphate buffer for 90-120 min in ice bath. Specimens then washed in distilled water then dehydrated in ascending series of alcohol (50, 70, 90, and 100%) then cleared in proline oxide. Specimens then embedded in Epon-812, then sectioned using ultramicrotome provided with glass knifes with an angel of 55, with thickness ranged between 1-2 µ, put on slides in 60 c for flattening .Then sections were stained in Toludin blue, mounted in DPX and examined and photographed by self-built camera.

RESULTS AND DISCUSSION

The body wall of *A. galli* consists of three layers namely cuticle, subcuticle or dermis and a muscle layer. In this basic layers it resemble most nematodes studied (Chitwood and Chitwood, 1974; Nawab-AlDuin, 1994; Decraemer et al., 1996; Hyman, 1951; Roberts and Janovy, 2005).

Cuticle

Cuticle in the present worm, *A. galli*, which is revealed by light microscopy found to consists of three layers namely: cortex or the upper layer stained deeply with Toludin blue 1.7 µ in thickness ,then middle layer or matrix 3.15 µ in thickness with no distinct constitutions (Figures 1 - 3), middle layer or matrix homogenous layer 5.65 µ in thickness , and fiber layer which in turn consist of several very thin layers , 5.65 µ in thickness arranged around the worm and settle on the basement membrane. Cortex show small round or oval pores connected by small canals (Figure 4).

The cuticle which cover all the body invaginate in different regions as mouth, anus, vulva, rectum, cloaca in addition also to cover the sense organs such as amphids, phasmids, and some time evaginate to cover some other sense organs such as cephalic, labial and caudal papillae. The thickness of cuticle differs in different body regions as the external cuticle which is usually thicker than the internal cuticle. In addition structure of cuticle differ in different regions for example matrix or the middle region in some sense organs and the cortex and basal layers not persist. It is believed that such lost help in accelerating transmission of nerve impulses to nerve endings underneath the thin cuticle as reported by Bird (1971) and as found in *A. lumbricoides.*

On the surface of cuticle of *A. galli* plugs a transverse

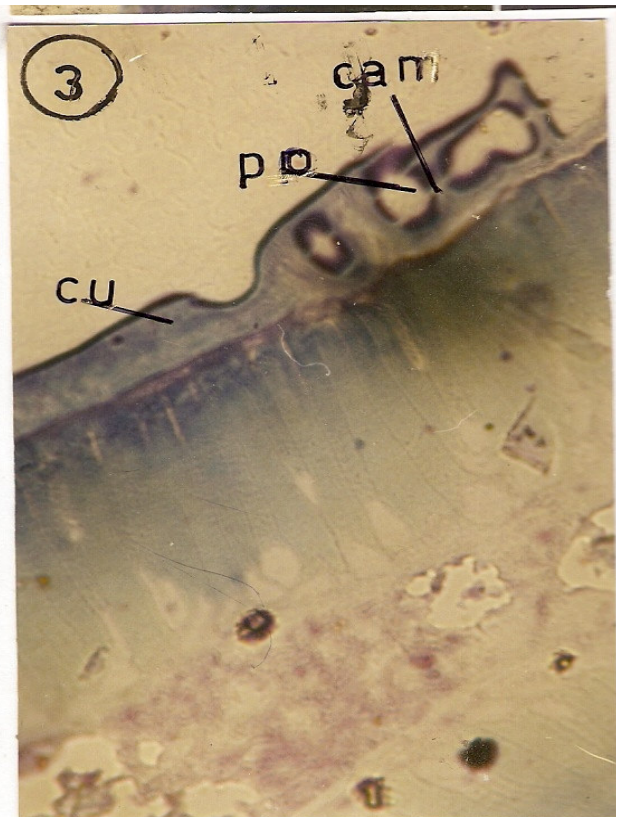

Figure 3. Photomicrograph of a transverse section in the body wall showing pore and canal system.
Can; canal, **po;** pore, **cu:** cuticle. TB ×400.

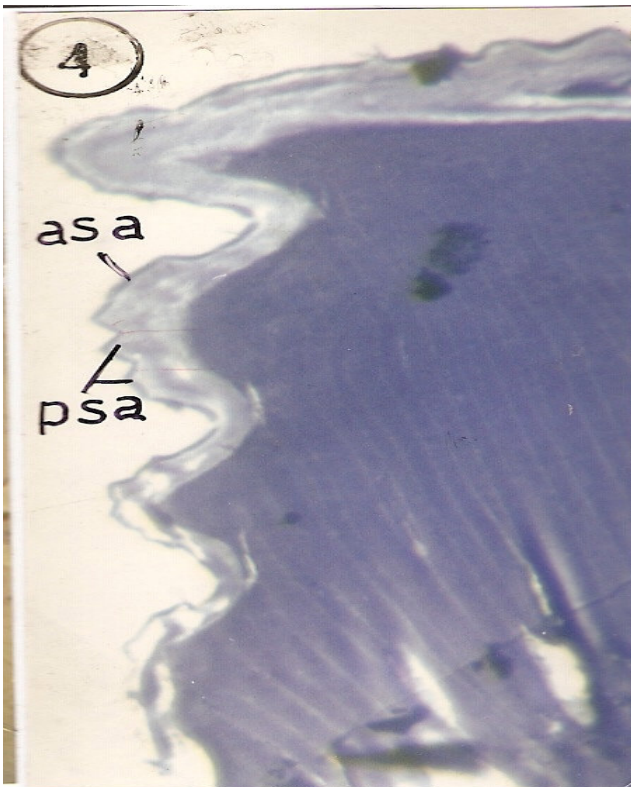

Figure 4. Photomicrograph of tangential section in the body wall showing transverse striations and annuli.
Asa; anterior subannulus, **psa;** posterior annulus. TB × 200.

annuli which divide the body into anunuli, the distance between each two 0.22 µ ,each annulus is subdivided by three subannuli, large anterior 0.124 um in long, and the other two are small, middle 0.047-0.054 µm, and the posterior 0.047-0.054 (Figure 4). Similar typical transverse striation was also observed in *Syphacia caudibandata* sp. by Ghazi et al. (2005). Similar observation was noticed by Decraemer et al. (1996) in free living nematodes in *Criconema parodoxiger*. In the present worm, striations mostly uniform in body while in *Pseudomazzia macrolabiata* are more prominent posteriorly as found by Bilquees et al. (2005). Sometime platelets or scales could be observed in other species (Jairajpuri and Southey, 1984). It is possibly that these rings appear as a result of fast cuticle deposition during ecdysis or during formation of new cuticle. Furthermore extensions from the cuticle of the present worm in the anterior and posterior regions of this worm especially in male forming cervical alae (Figure 5), and caudal alae (Figure 6).

The cuticle of *A.* lumbricoides found to compose of collagen which is secreted by epidermis and this layer is unique in nematodes (Ruppert and Barnes, 1994). Citwood and Chitwood (1974) reported that *Ascaris suum* cuticle is composed of five layers of protein namely albumin, glycoprotein(mucoid), fibroid, and collagen and the albumin ratio is 25%, glycoprotein undetermined fibroid 35% while the collagen constitute 29% while keratin 2.2%. Croll (1976) believed that the cuticle is 75% of water and remaining part is protein and little quantity of carbohydrate and fat. In more recent reference it is believed that the outermost covering of nematode body is a non-living proteinaceous cuticle (Shimk, 2008). This protein is collagen which is the major constituent of vertebrate ligaments, and is not elastic and not stretchable. This collagen is basically three layered-structure secreted tightly adjacent to one another giving strength and resiliency to the body wall (Shimk, 2008).

Cuticle thickness differs in different nematode species. In the present worm thickness is ranging from 8.5-11 µm while in plant parasitic nematodes or free living nematode it is much lower, 10-13 nm (Lee 1970; Bonner and Weinstein, 1972; Anderson, 2000).

Bonner and Weinsein (1972).) found that the cuticle thickness in Nippostrongylus brasiliensis reach about 60 nm. Also, thickness differ according to sex as found in the present worm 8.5 and 9.5 µ in male and female respectively. The results coincide to that found in *G. boophthorae* as the cuticle thickness is 3 and 4.5 um in male and female respectively (Batson 1979). Also results are similar to those found in *Rhabdochona tigrae* a para-

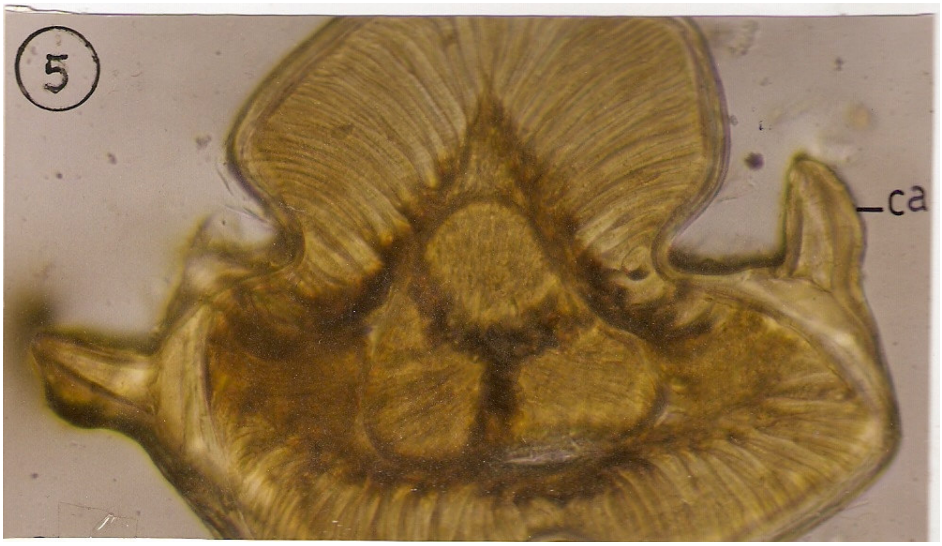

Figure 5. Photomicrograph of a hand section passing through oesophagus showing cervical alae (ca). ×200.

site in freshwater fishes as its cuticle measure 1.3 and 3.29 um in male and female respectively 9Nawab-AlDuin, 1994). Such thickness of cuticle in female possibly because it usually posses a double reproductive organ with two uteri filled with eggs as such need protection from external environment.

Cuticle thickness depends mainly on the size of the worm, it is usually thicker as the worm is larger in size, but a ratio is taken between thickness of cuticle and diameter of the worm the condition is reverse, this indicate requirement of thick cuticle in size . The present worm ratio is 1:75 in males and 1:79 in females while in *A.* lumbricoides 1: 100, in *Ancylostoma dudenale* it is 1:51 (Nawab-AlDuin, 1994), accordingly the ratio in *A.* lumbricoides is considered low if compared to the diameter of the worm, similarly R. tigrae.

Curiously, the thickness of cuticle in the present worm, *A. galli*, is thicker than the plant parasitic species, the free living form close to animal parasitic forms as such may be thickness here is a protective tool against digestive enzymes usually present in the alimentary canal of the hosts, and possibly cuticle also secrete some materials to neutralize the alkaloid medium of the intestine.

The thickness of cuticle in the anterior portion of the present worm, *A. galli*, is more than the middle of the body, a phenomenon which is similar to that reported by (1) in *A. duedenale*, it is likely because the anterior and posterior portions have less diameter than the middle. It is similar to the results of Nawab Al-Deen (Nawab-AlDuin, 1994). observed in R. tigrae, she postulated that thickness in anterior and posterior regions is due to thickness of cuticle constitutions. It is possibly also that the thinness of cuticle in the middle region of the worm is due to stretching effect due to the bulk of reproductive organs in the middle of the worm.

In the present study the cortex of *A. galli* appear as one layer with 3.15 µ in thickness and many structures appear as rounded or oval pores or canals connected together by small canals. In ultrastructure study the cortex has two layers, external and internal, the external layer is with more contact with the environment as such this layer is thin and porous as in large ascarid worms (Bird and Deutsch 1957; Watson, 1965). In this situation Tim (1949). suggest through his long experiments on A. lumbricoides that this layer has a permeability to drugs and has very thin fatty layer on the surface of cuticle. Furthermore, Bird (1957) found that the cortex has branched network of canals and pores and has a thin fatty layer measure about 0.1 µ. On the other hand, internal cortex differs in its thickness in different nematodes and is fibrillar in nature (Bird, 1971). Furthermore, this layer in large worms contains transverse structure underneath the transverse furrows which are present on the external surface of the worm which separate the external annuli. Researchers gave many terms to these organelles such as circular lamellae, fibers, strands of condensed materials, pore canals, or thick fibrous masses (Bird, 1971). In the present investigation the best name to be given to these organs are canals or pores as recovered in A. galli. It is likely that these canals and pores make an exoskeleton and pores that materials passing through to the exterior vastly (Bird, 1971). Watson (1965) during his study on *A. lumbricoides* observed that these canals and pores are connected canals and can reach down to the basal fibriliar layers. On the other hand, Inglis (1964) believed that all connecting canals observed in nematodes depends on system of punctuation canal as a system is evolved to allow the growth by addition of new materials by the epidermal protrusions. Anya (1966a) discovered the presence of

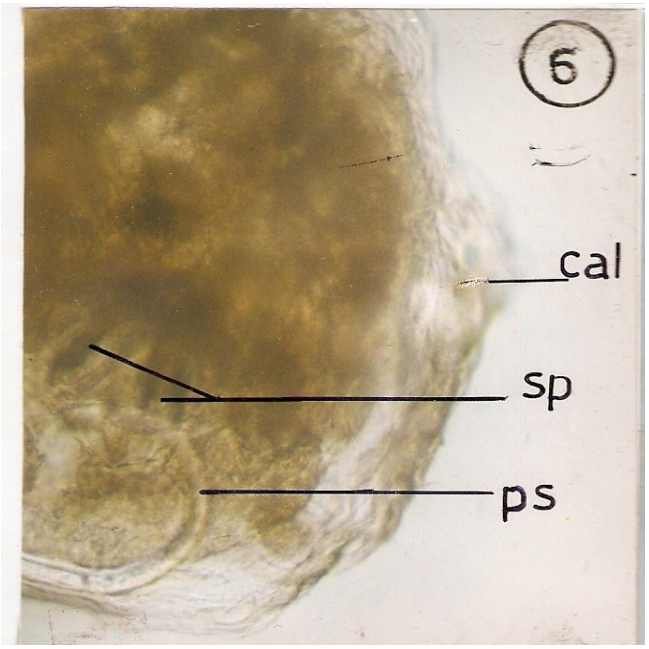

Figure 6. Photomicrograph of a hand section passing in the posterior end of the male showing caudal alae and precloacal sucker.
Cal; caudal alae, **sp:** spicules, **ps;** precloacal sucker. × 40.

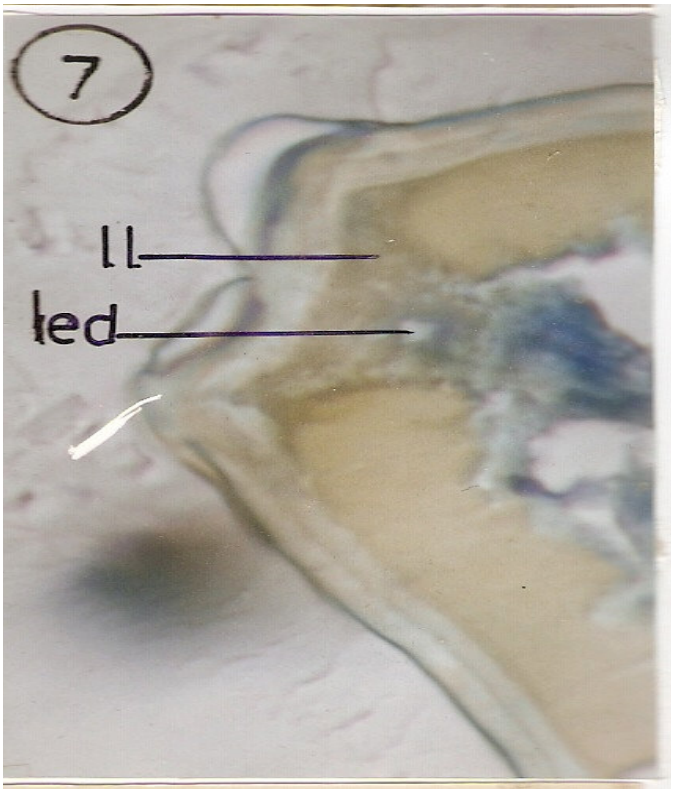

Figure 7. Photomicrograph of a section in the body wall showing excretory canal.
LI; lateral line, **led;** longitudinal excretory duct. TB ×400.

RNA and ATPase, acid phosphates, and ascorbic acid in the cortex of three nematodes studied including *A. lumbricoides*, while in other of study, Anya (1966b) concluded that the cuticle is able to synthesize protein which it needs.

In the present worm, the middle layer or the homogenous layer is lacking structures and its thickness is 1.7 µ. This lacking is similar to that found by Watson (1965) in large ascarid nematodes.

In other nematodes this layer is not homogenous layer as it is supplied by struts or skeletal rods filled with hameoglobin and are connected by collagen fibers present in this present layer. As concern chemical nature of this layer it consists of proteins which is similar to collagen as well as non-specific esterase and acid mucoplysaccaride and a few lipids but this layer has no metabolic activity such as cortex (Bird, 1971).

The innermost layer of A. galli is the fibril layer which appears to be consists of several thin layers arranged around the worm and its thickness is about 5.65 µ. This layer has been described by many researches and has been given different names such as striped layer with regularly arranged rods or canals regularly spaced striations or regularly arranged crystalloid structures (Bird, 1971). In the present worm, *A. galli,* this layer seems to be close to striped layer. It is obvious from other researches that this fibrilar layer is more prominent in worms suffer from changes in different habitats as found by Lee (1993) that the reasons of the presence of regular spaces between fibers is likely depending on its chemical nature as it is formed from protein with closely bands in its molecules which became more firm in habitat changing especially in plants parasitic nematodes such as Meloidogyne javanica.

The hypodermis of this worm, *A. galli* is syncial type, and its nuclei are constricted to the longitudinal cords in the thickening of hypodermis that is, dorsal, ventral, and two lateral cords. Mainly these nuclei are present in the lateral cords and the longitudinal excretory canals pass through these cords (Figure 7) This finding is similar to those found by Hinz (1966) Paraascaris equorum and Davey (1965) in Phocanema decipens and Watson (1965) in *A.* lumbricoides and Rogen et al.(1967) in X. index.

In the present worm the hypodermis is thin and not developed, hypodermis become thicker as proceed posteriorly to reach 2-3 µ in thickness in the inter-caudal region as well it become thicker in the four longitudinal cords protruded in the pseudocoel and reach maximum thickening (growth) near the nerve ring. Moreover, in addition to the main four longitudinal cords which divide the muscle bundles in four groups there are another four secondary cords which divide the muscle groups into 8 sectors (Figure 8).

This is similar to that found by Roggen et al. (1967) in *X. index* as they found that the hypodermis is very thin

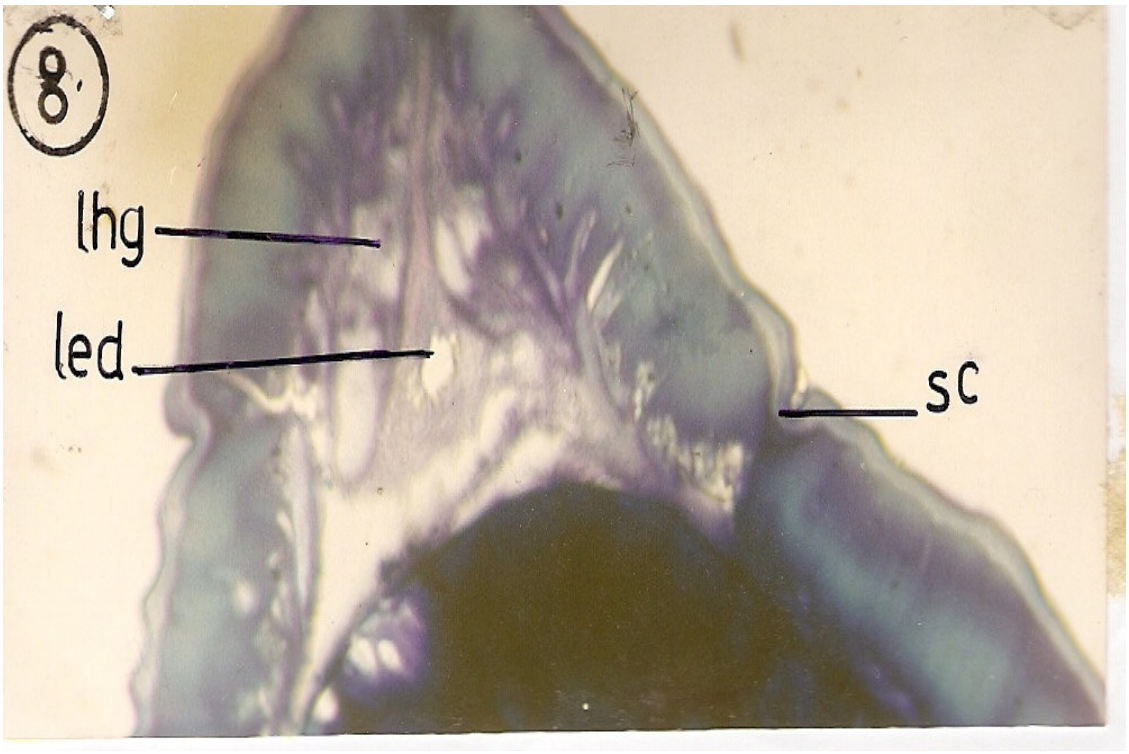

Figure 8. Photomicrograph of a section passing through oesophageal region showing secondary striations (constrictions) in the body wall.
Lhg; lateral hypodermal gland, **led;** longitudinal excretory duct**, sc;** secondary cord. TB × 400.

lie underneath the cuticle and also has four small longitudinal cords devoid of nuclei in the anterior region, and the best growth of ventral, dorsal, two lateral cords in just posterior to the nerve cord. Batson (1979) found that hypodermis in *G. boophthorae* is very thin and its thickness is 0.5 um in the inter-cordal region and became thickened in the four longitudinal cords.

Nuclei are present in epidermis of the four main cords of the present worm, *A. galli* especially in lateral cords. This phenomenon is similar to those observed in *A. lumbricoides* and *P. equonum*, *P. decipens* (Bird, 1971).

As concern lateral cells in *A.* galli present in lateral cords especially in the region posterior to the esophagus in the middle of the body are larger and more prominent and are continuous just before the posterior end of the body (Figure 9). These cells are present in the form of pyriform clusters, lateral cells, clup-shaped. These cells enlarges towards the pseudocoel and each posses large nucleus forming lateral hypodermal glands .The depth of these glands are 97(78-101) µ in male and 148(112-126) µ in female (Figure 10). Furthermore, three spines protruded from the surface of the cuticle situated near the opening of the hypodermal glands (Figure 11). From each canal arises two canals are united to open with small lateral pore underneath the cuticle to protrude in space situated underneath the cuticle in which open the lateral glands. These protrusions might help in the movement of materials thrown in the space or transport some materials from cuticle. These cells and their ducts were noticed in some marine nematodes (Hyman 1951; Chitwood and Chitwood 1974). Birds (1971) reported the presence of proteins, nucleic acids in hypodermis and histochemical changes happen and structural change before moulting. On the other hand McKerrow and Huima (1999) indicated that the hypodermis is main protein synthesis site which supply the hypodermis itself and the cuticle.

In the present study the hypodermal cells present in the four main cords are bulged into the pseudocoel through out the body very close to the alimentary canal (Figure 10) which mean a possible transport of nutrients to this canal. Furthermore, hypodermis is syncytial which gave one path with resistance of cell membranes for the transport of protein in the bodies of hypodermal cells. This conclusion is similar to that given by McKerrow and Huima (1999). Furthermore, in the present, *A. galli*, lateral glands were larger in female than male, it may be because the female deposit large number of eggs and require large amount of proteins.

Further study especially at molecular level, as performed by Kampfer et al. (1998) and Litvaitis et al. (2000), such as phylogenetic analysis of rDNA sequences, nucleotide sequences of D3 expansion segments (26/28s rDNA) can reveal more about the

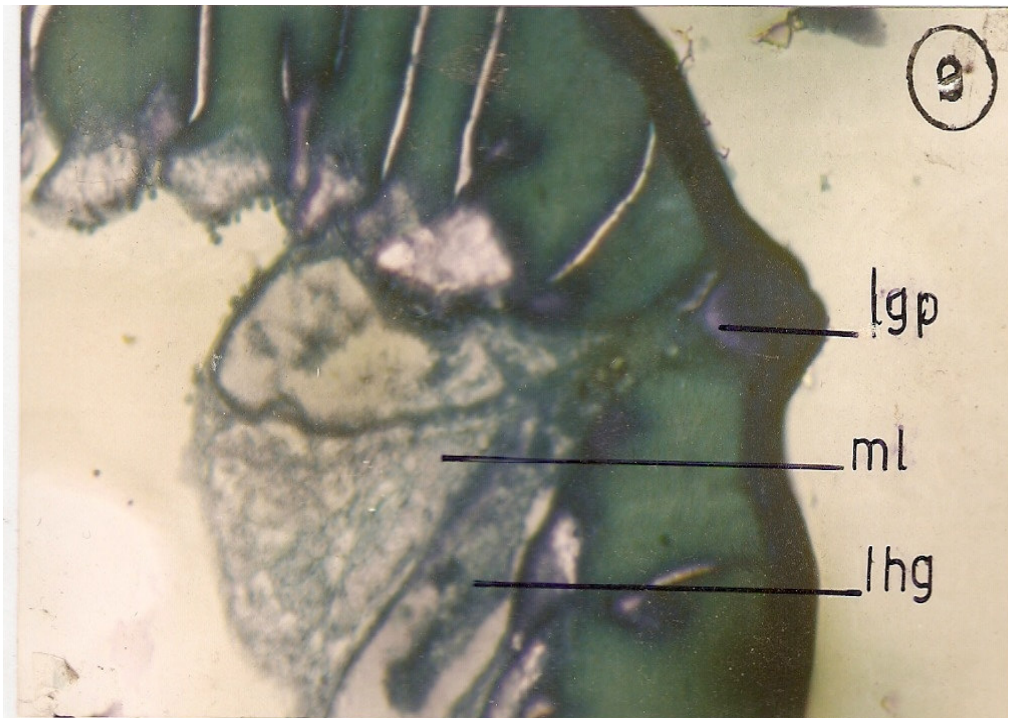

Figure 9. Photomicrograph of a section passing in the oesophagea region showing lateral glands. **Lgp;** lateral gland pore, **ml;** median line, **lhg;** lateral hypodermal gland. TB × 1000.

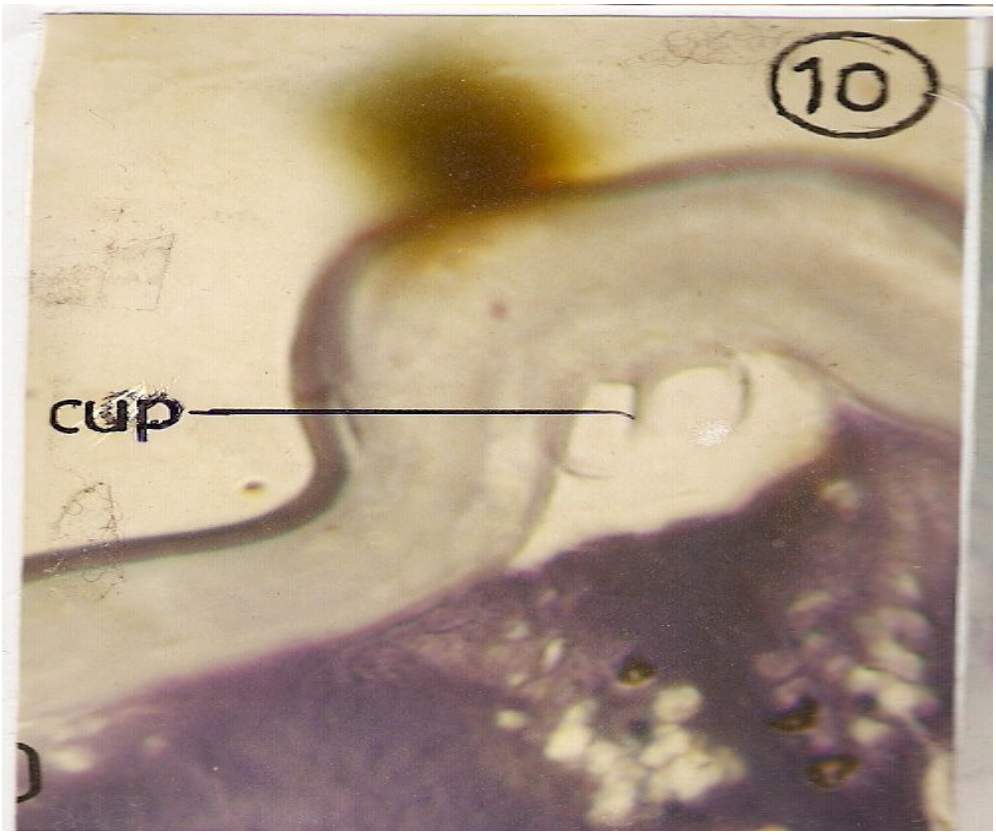

Figure 10. Photomicrograph passing in the body wall showing cuticular processes near the open Cup; cuticular process .TB × 1000.

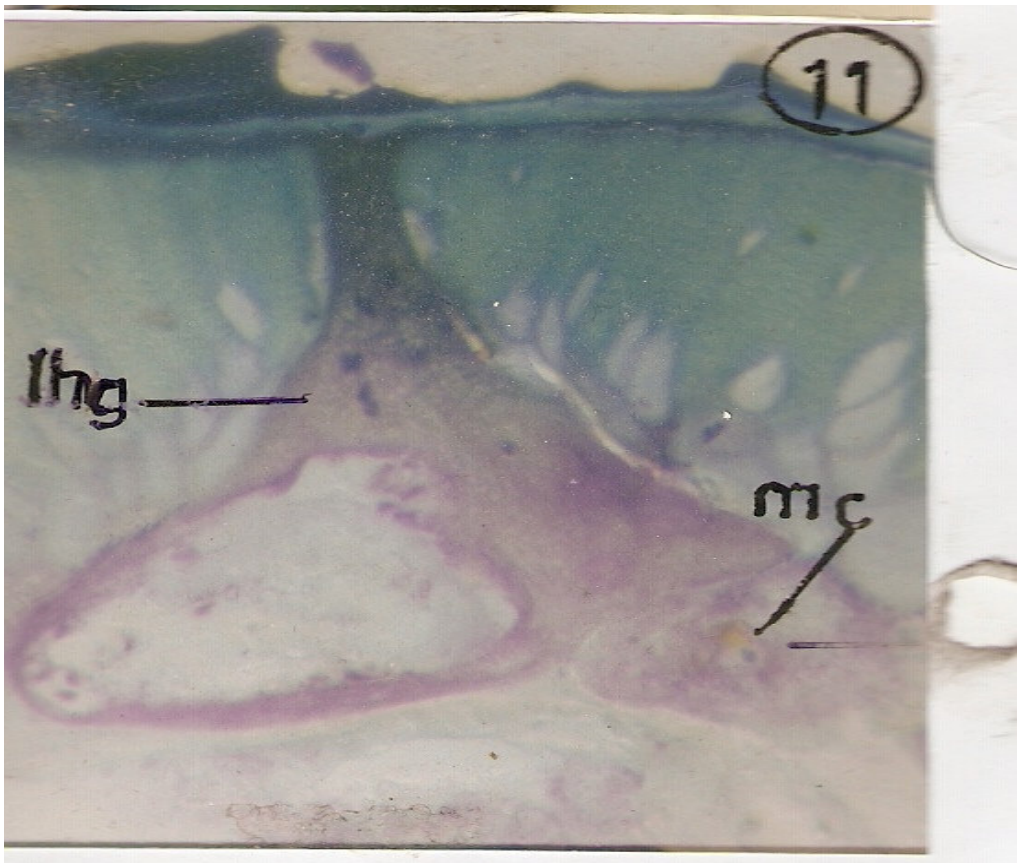

Figure 11. Photomicrograph of a section passing through the body wall showing lateral gland and its nervous supply.
lhg; lateral hypodermal gland. TB × 400.

different body structures of the present nematode in addition to tracing its affinities among other ascarids nematode to explore its diversity.

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