THE STRUCTURE AND CHEMICAL COMPOSITION OF THE BODY WALL OF ANIMAL-PARASITIC NEMATODES

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ABSTRACT

The nematode body is enclosed by a body wall comprising of three different layers. The outermost being a thick multilayered cuticle underlined by the middle hypodermal layer pronounced at four regions in the form of dorsal, ventral and a pair of lateral hypodermal cords. The innermost layer is constituted by a single layer of longitudinal muscle cells. Although the outer envelope of nematodes has been studied by a host of workers yet the controversy regarding certain aspects of its structure, chemical composition and function still prevail. The presence of proteins, carbohydrates, lipids, acid mucopolysaccharides and a variety of enzymes have been detected by various workers in different species of larval and adult nematodes. Since the mere survival of a nematode parasite in the body of its host is mainly attributed to the uniqueness of its body covering, hence the study of its nature and chemical composition is of paramount importance in parasitic nematology. The present paper briefly reviews the histological and histochemical studies on the body wall of animal nematodes parasitic animals.

INTRODUCTION

Nematodes are the most numerous multicellular animals present on the earth (Maggenti, 1981). They parasitize man, livestock, crops and have a deleterious effect on all. Not only is the economics of nematode parasitism important but nema themselves are a fascinating subject for the study of morphology, biology and host-parasite relationship. Morphologically the Nematode is an exceedingly variable group and there hardly exists any statement that could be made regarding their histomorphology, which would apply to all forms. Different types of anatomical features are present in different species of parasitic nematodes which are the result of adaptation influenced by their particular type of habitat (Chitwood and Chitwood, 1950).

Some recent studies on the body wall of animal parasitic nematodes have been performed by Johal (1995), Johal and Shivali (1996), Page (2001), Cano-Martil *et al* (2006), Rahemo and Hussain (2009) and Lalchhandama (2010). The multilayered nature of the cuticle had long been established with the number of layers varying from two in the larva of *Trichinella spiralis* (Beckett and Boothroyd 1961) to eight in adult *Oxyuris equi* (Bird, 1958). Since then there has been a general agreement on the basic terminology for the nematode cuticle as a three-layered structure consisting of cortical, median and basal layers. The nematode cuticle has been comprehensively reviewed from time to time by Maggenti (1981), Bird (1980 and 1984), Wright (1987), Bird and Bird (1991) and Page (2001). The nematode cuticle nearly always consists of at least two major layers, the cortical and the basal. However, an exception to this general rule was found in the insect parasitic nematode *Bradynema* spp. where the normal cuticular structure has been replaced by microvilli (Riding, 1970). The two-layered cuticle characterized the adult females of the family Heterodidae (Bird and Rogers, 1965; Kampfle, 1966) and the parasitic larvae of *Trichinella spiralis* (Beckett and Boothroyd, 1961). This two-layered structure was believed to be due to rapid periods of growth at some stages in these animals. However, in pre-parasitic larvae and males of Heterodidae and in the adults of Trichuroidae the normal three-layered cuticle prevailed (Sheffield, 1963 and Wright, 1968).

RESULTS AND DISCUSSION

Structurally, the nematode cuticle is quite diverse, not only in different genera and families, but also within species. There may even be marked differences in the cuticle of male and female worms and in the different body regions of the same individual. These differences in the cuticle can be more marked in parasitic nematodes. (Cano-Martil *et al*, 2006).

In *Oxyuris equi*, the cuticle consisted of an outer and an inner cortical layers, a fibrillar layer consisting of two distinct layers of fibres, a homogenous layer and two distinct layers of fine fibres, a homogenous layer and two fibre layers (Martini, 1912). Bird (1958) studied the cuticle of *Oxyuris equi* and described an additional fibrillar layer under the cortical layer which was not mentioned by Martini (1912). The cuticle of *O. equi*, thus comprised eight layers, starting from the outermost layer inwards: (1) an external cortical layer; (2) an internal cortical layer; (3) a fibrillar layer; (4) a part of homogenous layer; (5) and (6) two outer fibrous layers; (7) and (8) two inner fibrous layers. There was no indication of presence of any bounding layer or a basal layer.

Lee(1965) reported that the cuticle of adults of *Nippostrongylus brasiliensis* had three main layers, an outer cortex, a middle matrix and an inner fibre layer. The cortex appeared to be a single layer which

varied in thickness, being thin at the apices of the longitudinal ridges and much thicker between the ridges. The cortex was separated from the fibre layer by a fluid filled region, the middle layer. The fibre layer was found to be composed of two fibre layers and a basement membrane. Using light and scanning microscopy, Anya (1966) observed that the cuticle of *Aspiculuris tetraptera* had three basic layers namely, the cortex consisting of an outer and inner cortical layer, middle matrix and inner fibre layer which in turn is made of three layers. In addition, a thin osmiophillic superficial membrane on the surface of the cuticle, discernible only with the electron microscope, was reported.

Tomita (1975) described the cuticle of *Thelazia callipaeda* to be composed of two layers, an external layer consisting of three sub-layers forming the transverse body striations and an internal layer composed of six sub-layers. Franz (1980) made scanning and transmission electron microscopic studies on the cuticle of *Onchocerca volvulus* and found it to be consisting of a cortical, a median and a basal layer. The outer limitation of the cuticle was an irregularily folded surface membrane, which showed a regular honey comb pattern and covered the entire worm with the exception of anterior and posterior ends. Lee and Nicholas (1983) used plasma etching to reveal the structure of the cuticle of adult *Nippostrongylus brasiliensis*. The cuticle revealed an outer membrane like layer, a single cortical layer, a fluid filled layer, two fibre layers and a basement lamella.

Martin and Lee (1983) described the structure of body wall of adult *Nematodirus battus*. The cuticle of the male *Nematodirus battus* was divided into three main layers namely, an outer cortex, a middle layer and an inner fibre layer. These three major layers were further divided into eight distinct layers, namely,an outer membrane like layer or epicuticle ; a narrow electron dense outer cortical layer; an inner cortical layer a matrix layer; three fibre layers and an inner fibrous basal lamella. The basal lamella possessed local areas associated with hemi-desmosomes on the hypodermal membrane. The cuticle of the anterior half of the body of female was found to be similar to the male while the posterior half was enveloped by seven-layered structure consisting of an outer membrane like layer, a narrow outer cortex, an inner cortex, a thin matrix layer, two fibre layers and a fibrous basal lamella.

Based on their ultrastructural and cytochemical studies, some authors (Lee, 1965 and Bird, 1980) considered that the outer covering of the cuticle was a "cell membrane" and referred to it as an epicuticle. Since then the use of terms such as "membrane", "membrane-like", "modified membrane" for different regions of the cuticle's surface have created certain confusions and implied various concepts regarding its composition and function. Zuckerman *et al* (1979) and Bird (1985) used the term "glycocalyx" for the carbohydrate moieties on the outermost surface of the cuticle which sometimes appeared as a fuzzy coating. The presence of carbohydrate moieties and use of the term "glycocalyx" for the surface membrane of the cuticle gave rise to the impression of epicuticle being a cell membrane. This concept of the outside layer being a cell membrane had been discussed by Wright (1987) who pointed out that the surface structure was likely to vary considerably in different nematode species as a result of different functional and anatomical requirements. To solve this problem and minimize further confusion, he suggested that, it will be much appropriate to use the term surface coat for the outer carbohydrate component of the cuticle rather than the glycocalyx. He also suggested that the epicuticle was not a cell membrane, however, with studies it could

be seen as a surface trilaminate differentiation containing lipids.

Nevertheless, the term epicuticle now appears to be accepted for the outside layer. Regarding remaining layers of the cuticle, there seems to be a consensus that a generalized cuticle would consist of an epicuticle, a cortical layer, a median layer and a basal layer (Bird and Bird, 1991). Fok *et al* (1992) described the cuticle of *Nippostrongylus brasiliensis* as a three layered structure and the outer part of the cuticle, the epicuticle, was found to be covered with a polyanionic coat, the glycocalyx, which was stained by the anionic dye ruthenium red. Lee *et al* (1993) studied the structure of the cuticle of adult *Nippostrongylus brasiliensis* by freeze-fracture technique and transmission electron microscopy. The epicuticle cleaved readily to expose E- and P- faces. The P- face of the cuticle possessed a small number of particles, similar to intra-membranous particles, whilst the E-face contained a few widely scattered depressions. Despite the presence of these particles the epicuticle could not be considered as a true membrane. Freeze-fracturing of the remainder of the cuticle confirmed its structure as described by conventional transmission electron microscopy.

Since then a number of workers have authenticated the modern perspective regarding the nature and structure of the cuticle evidenced by their studies on larval stages of various nematodes. Ultra-structural analysis of the cuticle of *Brugia malai* micrifilarae indicated that it was composed of two regions: the inner one with a homologous aspect and the outer one designated as the epicuticle. Further, three laminae separated by electon- lucent regions were seen in the epicuticle (Araujo *et al*, 1995). Replicas of freeze-fractured micrifilariae showed the presence of two fracture planes in the epicuticle and no fracture in the inner region of the cuticle. The P-face of the epicuticle outer fracture plane presented a large number of densely-packed small particles and many protuberances.

Neuhaus *et al* (1996) investigated the ultrastructure, development and morphogenesis of the body cuticle of adult and juvenile *Oesophagostomum dentatum* by light microscopy, scanning electron microscopy and transmission electron microscopy. The cuticle of the first three juvenile stages was consisted of a trilaminate epicuticle, an anormous layer and a radially striated layer. In the last juvenile stage and adult worm, the radially striated layer was replaced by a fibrous layer with three sublayers of giant fibres and a basal amorphous layer. Consequently, the body cuticle of adult *Oesophagostomum dentatum* revealad three basic layers: an epicuticle, a three-zoned amorphous layer and a three-zoned basal fibrous layer.

Martinez and Souza (1997) examined the cuticle of the infective third stage larvae of *Strongyloides venezulensis* by transmission electron microscopy, ruthenium red cytochemistry and deep-etch techniques. The cuticle was seen to be consisting of five layers: epicuticle, cortical, medial, fibrous and basal. The epicuticle had a trilaminate appearance and the surface coat stained with ruthenium red. At the level of cortical, median and basal layers, interconnecting fibres and globular structures were seen. The fibrous layer was formed by parallel bars of thick fibrous elements. Patel and Wright (1998) had homologized the different nomenclatures of the cuticle given by earlier workers. They divided the cuticle into four layers: epicuticle, a combined cortical and median layer, stratified layer and the fibrous mat respectively, corresponding to the terms surface sheet, cortex mat, striation band and fibrous mat used earlier by Jackson and Bradbury (1970) and the terms epicuticle, cortical, median and basal

layers respectively described by Kondo and Ishibashi (1989). Furthermore, Patel and Wright (1988) also followed the nomenclature for defining the different layers of the cuticle as a modification of the commonly accepted three - layer plan. The layer described as a basal layer by Bird and Bird (1991) was referred here as striated layer and fibrous mat. The layer named as fibrous mat was found to be analogous to the structural layer referred by Maggenti (1981) as the endocuticle. The epicuticle appeared as a closely applied electron dense layer but it is not possible to observe the trilaminar structure. An indistinct external layer was observed and this was assumed that this represented the surface coat. The cortical and median layers were observed as one homogenous zone.

Cano-Martil *et al* (2006) light microscopic and transmission electron microscopic studies on the cuticle of adult *Diplotriaena tridens* and described three major zones namely, cortical, medial and basal zones. This trilayered stratified structure is externally covered by a trilaminar epicuticle.

Rahemo and Hussain (2009) studied the body wall of *Ascaridia galli.* The cuticle was found to be consisting of three layers namely, outer cortex, middle matrix and inner fibre layer. The fibre layer in turn consists of several layers of dense tissue. They also concluded the middle layer of the cuticle as a homogenous layer without any struts. The cuticle of *Ascaridia galli* was found to be a complex layer of proteinaceous rings forming a hard and thick unit and is composed of several discrete concentric layers running continuous around the body (Lalchhandama, 2010).

Although the cuticular layers vary in thickness but the average ratio of thickness of the cuticle to the diameter of the nematode was considered to be about 1:30 to 1:40. Exceptions to this were found in large ascarids (1:100) (Bird, 1971) and *Ancylostoma* (1:10) (Loss, 1905). The cuticle of *Strongylus vulgaris* being much thicker than that of *Oxyuris equi* and also thicker than that of *Ascaris lumbricoides* (Smyth, 1996).

Externally the cuticle revealed a variety of structures which were reviewed by Chitwood and Chitwood (1950). These include much prevalent transverse markings, longitudinal ridges and alae. Structure and pattern of the longitudinal ridges were discussed by a number of workers: Lee (1965), Lee and Nicholas (1983), Martin and Lee (1983) and Nembo *et al* (1993).

Lassaigne (1843) was the pioneer of performing chemical analysis on the cuticle of nematodes and from his observations, he established the chitinous nature of the cuticle in *Ascaris.* Subsequently, the presence of proteins in the nematode cuticle was also reported. Based on his extensive studies on the chemical composition of the cuticle of *Ascaris lumbricoides*, Chitwood (1938) come to the conclusion that the cuticle consisted of at least five different substances namely, albumin, glucoprotein, a mucoid, a fibroid, a collagen and a keratin.

The presence of proteins along with traces of carbohydrates and lipids had been reported in the cuticle of various nematode species by a host of workers: Bird and Rogers (1956), Beams (1964) and Anya (1964 and 1966). In *Toxascaris leonine*, Johnson (1968) observed the presence of proteins in all the layers except the fibrillar layer and the maximum concentration being in the cortical layer. Kan and Davey (1968) while working on *Phocanema decipiens* indicated that the rich protein content of the outer cortex was responsible for the high resistance offered to the passage of host enzymes in parasite body.

Jenkins (1970) observed the proteins with –SS- and $–NH_2$ bound groups in different cuticle layers of *Trichuris suis*, Singh and Khera (1972) attributed a collagenous nature to the cuticle of *Dracunculus medinensis*. The presence of different groups of proteins in all the seven layers of the cuticle was observed by Lestan and Dubinsky (1973) in *Porocaecum*. The presence of –SS- group was depicted in the outer layer, collagen in the fibrillar and basal layers and elastin in the median layer. The presence of –SS- and –SH group bound proteins was also reported in the inner cortical layers of *Enterobius vermiculuris* by Hulinska and Helinsky (1973).In *Paranisakis kherai*, the cuticle was found to be rich in proteins bound with –SH, -SS- and -NH2 groups, which were distributed in moderate quantity in the outer and inner cortical layers and in high concentration in the matrix layer (Gupta and Garg, 1976). A substantial amount of protein (collagen) was also observed in the outer cortical and fibrillar layers of the cuticle of *Pseudoproleptus kherai* by Sheikher and Garg (1979). Ouazana (1982) stated that the collagen formed the major constituent of the nematode cuticle. Johal (1988) too, described the presence of collagen like fibres in the basal layer of the cuticle of *Oesophagostomum columbianum*.

By using immunostaining technique, Takahashi *et al* (1993) studied the ultrastructural localization of phosphorylcholine (a hapten) from the inner layers of the body cuticle of *Trichinella spiralis*. Majumdar *et al* (1996) reported that the cuticle of *Ascaridia galli* was basically proteinaceous with a rich quantity of elastin but poor quantity of $–NH_2$ bound proteins. Some tyrosin containing proteins were also located in the epicuticle region. The presence of chitin in the pharyngeal cuticle of *Oesophagostomum dentatum* was detected by Neuhaus *et al* (1997).

Carbohydrates were observed in the cortical as well as fibrillar layer of the cuticle in *Toxascaris leonina* by Johnson (1968). Gupta and Kalia (1978) reported that the polysaccharides with 1:2 glycol group together with glycogen were present in high concentrations in the matrix as well as in fibrillar layers of *Setaria cervi*. The presence of acid mucopolysaccharides on the surface of cuticle was observed in *Diplotriaena tricuspis* (Wajihullah and Ansari, 1981), *Oesophagostomum columbianum* (Johal, 1988) and *Toxocara canis* (Page *et al*, 1992). Majumdar *et al* (1996) described the presence of hexose containing mucosubstances in the epicuticle- exocortex of *Ascaridia galli*.

Concentration of lipid was reported by Gupta and Garg (1976) in the matrix and fibrillar layers of the cuticle in *Paranisakis kherai.* Prasad and Guraya (1977) reported that the bound lipids were present in the outermost surface layer of cuticle and bound as well as free lipids in the outer cortical layer in *Ascaridia galli.* Only a small amount of lipid was reported in the cuticle of *Tanqua anomala* (Kankal, 1989). On the other hand, Majumdar *et al* (1996) detected a substantial amount of lipid in the epicuticle- exocortex and a moderate amount of the same in the median zone of the cuticle of *Ascaridia galli.*

The metabolically active nature of the cuticle was determined by the presence of enzymes such as esterase in the cuticle of *Ascaris* by Lee (1962). Localization of esterase along with ATPase and RNA in the nematode cuticle (Anya, 1966) further authenticated that cuticle is a site for the synthesis of cuticle enzymes. The presence of a number of enzymes viz. esterase, acid phosphatase and adenosine triphosphatase was recorded in the cuticle of *Trichuris suis* and *Haemonchus contortus* by Jenkins (1970) and Sood and Kalra (1977).

Anya (1966) was able to detect some RNA activity in the inner cortical of *Aspiculuris*

tetraptera, Syphacia obvelata and Ascaris lumbricoides. RNA in the matrix layer of the subcuticle of *Diplotriaena tricuspis* and inner cortical layer of *Oesophagostomum columbianum* was reported by Wajihullah and Ansari (1981) and Johal (1988) respectively.

The basic nature of nematode hypodermis was described by Martini (1903). A syncytial hypodermis was reported by Loss (1905), Martin and Lee (1983), Neuhaus *et al* (1996), Rahemo and Hussain (2009) and Lalchhandama (2010) in *Ancylostoma, Nematodirus, Ascaris* and *Ascaridia*. Presence of three rows of nuclei in the lateral and one to three in the median cords was reported by Hyman (1951) and she also described that this number is subjected to variation in different species. However, Chitwood and Chitwood (1950) attributed a cellular nature to the hypodermis as they observed the presence of two sublateral rows of unicellular hypodermal glands situated in the lateral hypodermal cords opening by short ducts through pores in the cuticle in some species of nematodes. Johal (1994) reported the presence of large hypodermal gland cells, having prominent nuclei, constituting the lateral hypodermal cords. These cells were found to communicate with the overlying cuticle by a large pore present in the centre of the cord. Neuhaus *et al* (1996) reported the attachment of hypodermis to the cuticle via hemidesmosomes. Electron dense fine fibrils were reported to be extending from the hemidesmosomes a short distance into the basal amorphous layer of the cuticle.

Von Kemnitz (1912) in *Ascaris* and Martini (1916) in *Oxyuris* reported that glycogen was stored in considerable quantity in the hypodermal layer of the body wall. The presence of both glycogen and fat in the lateral hypodermal cords of various nematode species have been reported by Fairbairn (1957), Anya (1964) and Singh and Khera (1972). In the infective larvae of *Onchocerca volvulus*, extensive accumulation of glycogen was observed in the hypodermis by Endo and Trpis (1997). Presence of lipids in the hypodermis of *Trichuris ovis, Setaria cervi*, and *Oesophagostomum columbianum* was reported by Jenkins (1970), Gupta and Kalia (1978) and Johal (1988) respectively. The localization of keratin, diffused phospholipids and scattered lipids was recorded in *Dracunculus medinensis* (Singh and Khera, 1972) and *Ascaris* (Reznik, 1971). An appreciable amount of proteins was also observed by Johal (1988) in the lateral hypodermal cords of *Oesophagostomum columbianum*. Proteins contained in the haemoglobin were reported by Smith and Lee (1963) and Okazaki *et al* (1967) in the hypodermal columns of *Ascaris lumbricoides*.

Yanagisawa and Maki (1978) described that certain species of nematodes revealed the presence of enzyme acid phosphatase in high concentration in the hypodermis. Acid phosphatase in the hypodermis was also detected by Sood and Kalra (1977). The metabolic activity of hypodermis was established by the presence of RNA in *Oesophagostomum columbianum* (Johal, 1988).

Schneider (1860) performed an exhaustive study on the somatic musculature of nematodes and proposed descriptive nomenclature to each different category seen. He classified the muscles into three types based on the shape of the muscle cells, namely, Platymyarian (flat type of cells with fibres on the base), coelomyarian (V- shaped type of cells with fibres extending on lateral walls) and circomyarian (cells in which fibres encircle the cytoplasm). Later, the same author (1866) gave the classification of muscle cells according to the number of rows present in each hypodermal area and introduced the terms, holomyarian (with no or two rows), meromyarian (two to five rows) and polymyarian (large number of

rows). Although this nomenclature has no taxonomic significance and was simply one of the convenience, but it is still not discredited. In *Nippostrongylus brasiliensis*, Jamuar (1966) and Lalchhandama (2010) described that each muscle cell consisted of two regions, a distal fibrillar zone and a sarcoplasmic zone containing mitochondria and nucleus. Orientation of muscle cells was described by Maggenti (1981), who concluded that the muscles aligned anywhere from directly parallel to some 45 degree off the parallel. When parallel the cells were staggered like bricks so that a spiral around the pseudocoel was produced. Complex twisting, bending and rotational movements in nematodes is the outcome of this orientation. The presence of desmosomes for the attachment of coelomyarian muscles of *Nematodirus battus* to the hypodermis was reported by Martin and Lee (1983). Large concentrations of glycogen granules in the non-contractile part of muscle cells were reported by Hirsch and Bretschneider (1937). Lee (1960) stated that the amount of glycogen in the muscle cells in the anterior region was less than that found in the posterior region. Anya (1964) detected the presence of glycogen in non-contractile part as well as myofilaments of the muscle cells in some female oxyurids. Wajihullah and Ansari (1981) in *Diplotriaena tricuspis*, Johal (1988) in *Oesophagostomum columbianum* and Takahashi *et al* (1988) in *Trichinella spiralis* established that glycogen is concentrated mainly in the sarcoplasmic zone of muscle cells. Kankal (1989) detected glycogen in both the contractile as well as non- contractile parts of muscle cells in *Tanqua anomala.* Storey and Ogbogu (1991) observed that though glycogen was the main constituent of the muscle cells of adult *Litomosoides carinii* but the same was found to be absent in the larval muscle cells. Endo and Trips (1997) detected extensive accumulations of glycogen in the muscles of the infective third stage larva of *Onchocerca volvulus.* Besides glycogen, Gupta and Kalia (1978) and Wajihullah and Ansari (1981) described the presence of mucopolysaccharides too, in the muscle cells of *Setaria cervi* and *Diplotriaena tricuspis*. Protein is regarded as main constituent of the contractile part of muscle cells in nematodes. In *Toxascaris leonina*, Johnson (1968) stated that the contractile part of the body wall was found to be richest one as far as the amount of proteins was concerned. The presence of an appreciable amount of proteins was also reported in the muscle cells by Jenkins (1970) in *Trichuris suis* and Johal (1988) in *Oesophagostomum columbianum*. Kankal (1989) described that the protein formed the chief structural component of the musculature of *Tanqua anomala*. Gupta and Garg (1976) and Johal and Shivali (1996) detected the presence of lipids from the fibrillar zone of muscle cells.

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