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Observation on the body wall of a *Trichostrongylid nematode Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898

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Research Article

ABSTRACT

The cuticle of *Haemonchus contortus* has on its outer surface a highly resistant epicuticle-exocortex complex possessing a substantial amount of acid mucopolysaccharides in the epicuticle and lipid in the outer cortical layer forming a permeability barrier. The longitudinal ridges found in both the sexes, pertaining to the anterior region of the body, are supported by tough resistant struts. The median layer of the cuticle from where these struts arise is well developed, whereas the basal layer is less developed and its striated nature is also not evident. Histochemically, the carbohydrates and lipids are found in high quantities in the cuticle in general. The protein is restricted to the outer cortical and median layers only. The hypodermal areas are positive for lipids and carbohydrates. Large amount of glycogen is present in the non-contractile part the muscle cells except the perinuclear spaces whereas the contractile portion contains large concentration of proteins. However, both these areas of muscle cells are negative for nucleic acid activity.

KEYWORDS

Body wall, Cuticle, Histology, Histochemistry, Nematoda, Haemonchus contortus.

INTRODUCTION

Morphologically Nematoda is an exceedingly variable group and there hardly exists any common statement that could be made regarding their histomorphology and histochemistry, which would apply to all forms (Chitwood and Chitwood, 1950). Pawlowski (1987) while addressing the 6th International Congress at Brisbane, Australia stated that there is a renewed interest in basic research which can fill the hitherto unexplained gaps.

Although the outer envelope or cuticle of nematodes has been studied by a host of workers yet controversy regarding certain aspects of its structure and function still prevail (Kennedy, 1991). 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 the nature and chemical composition of the cuticle is of paramount importance in parasitic nematology. The various aspects pertaining to the body wall of nematodes are discussed by Bird and Bird (1991), Page (2001), Thompson and Geary (2002), Alvarez *et al*. (2007), Mehlhorn (2008), Rahemo and Hussain (2009), Lalchhandama (2010) and Singh (2012a; 2012b).

The gastrointestinal nematode, *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898 commonly known as Barber's pole worm or twisted worm is a major pathogen of small ruminants throughout the temperate and tropical regions of the world and is a significant cause of production losses. It is a highly pathogenic blood feeder helminth that causes acute amaemia, edema (bottle jaw), diarrhoea and ultimately death.

Previously, the histomorphology and histochemistry of various organ-systems of *Haemonchus contortus* has been studied by Singh and Johal (1997), Singh (2000), Singh and Johal (2001a; 2001b; 2001c; 2004) and Singh (2012a: 2012b). In the present research paper, the structure of the body wall of *Haemonchus contortus* is analysed and compared with the previous literature on other nematodes studied so far. The present study describes many micromorphological and histochemical variations in the body wall of *Haemonchus contortus*, which can fill the hitherto existing gaps in information regarding this aspect. This histomorphological study and histochemical localization of various macromolecules will be of significance to understand the metabolic activities

and fundamental functional aspects of this endoparasitic trichostrongylid nematode of small ruminants. It can also form the basis in evolving chemotherapeutic measures against this serious blood sucking pathogenic parasite.

MATERIALS AND METHODS

The nematode *Haemonchus contortus* was extracted from the abomasum portion of stomach of sheep (*Ovis aries*). In order to remove debris, the nematode worms were washed in 0.85% NaCl solution. For histomorphological and histochemical studies, the worms were fixed in alcoholic Bouin's fixative and Carnoy's fixative, dehydrated in a graded series of alcohol, cleared in methyl benzoate and embedded in paraffin wax. The sections were cut at 7µm in transverse and longitudinal planes by using rotary microtome. The serial sections arranged on albuminised slides were stained. For the histochemical localization of carbohydrates, glycogen, acid mucopolysaccarides, proteins and lipids the following staining methods were used.

General carbohydrates were studied by Periodic acid Schiff's staining technique (McManus, 1948). Glycogen was detected histochemically by Best's carmine staining (Best, 1906) and acid mucopolysaccharides by Alcian blue (Steedman, 1950). Nucleic acids were detected by Gallocyanin chromalum (Einarson, 1951) and Methyl green pyronin Y (Kurnick, 1955) techniques. For the localization of proteins, Mercuric bromophenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa, 1953) were used. The histochemical presence of lipids was detected by Sudan black B staining (McManus, 1946) and Oil red O in isopropanol (Lillie and Ashburn, 1943). The slides were examined under the microscope and photo micro graphed.

OBSERVATION

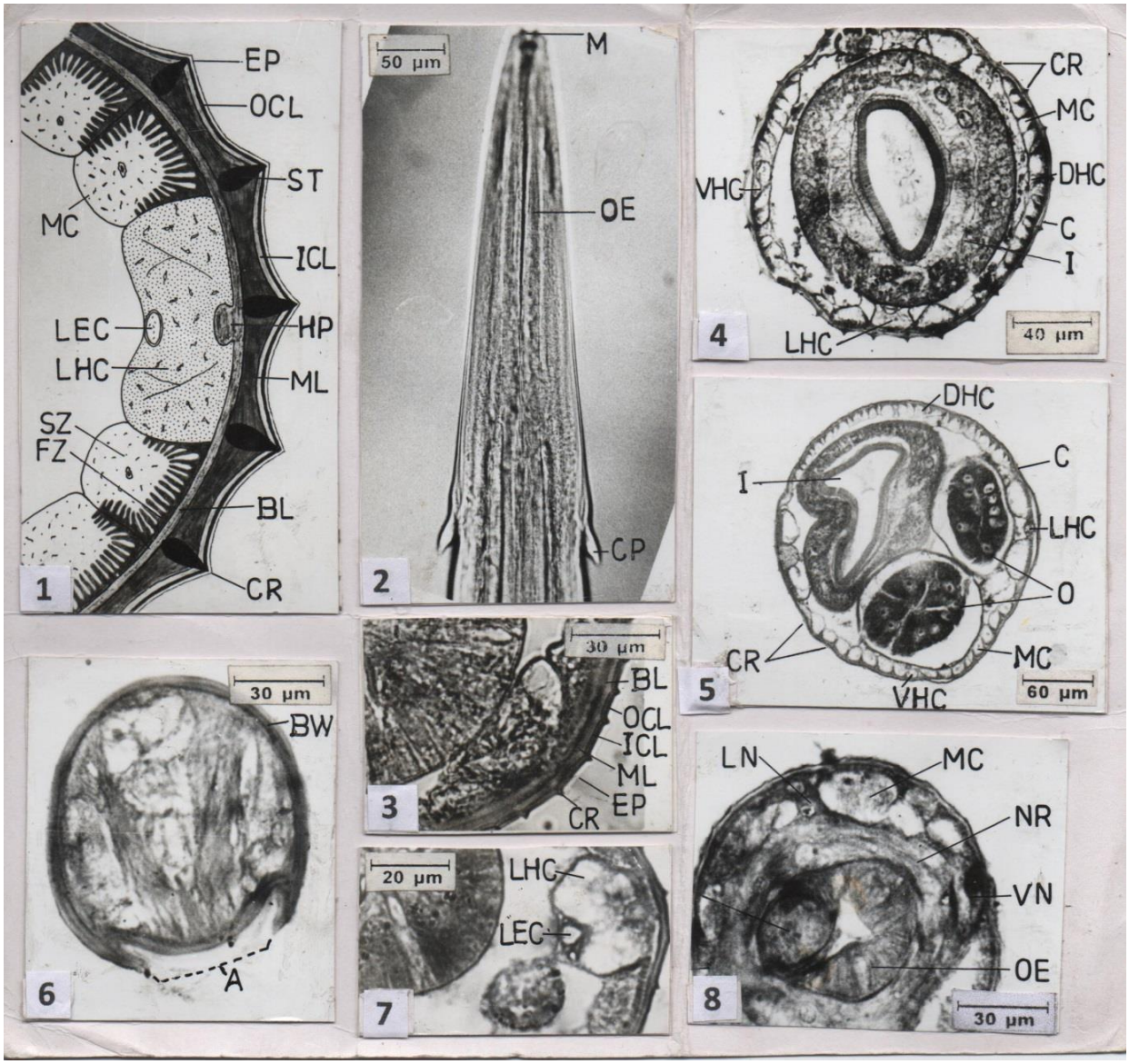
Structure: The body wall of *Haemonchus contortus* consists of an outer covering the cuticle, a hypodermis thickened at four places to form longitudinal cords and an inner longitudinal layer of muscle cells (Fig. 1).

Cuticle: The cuticle is a multilayered structure. It invaginates in the regions of mouth, anus, excretory pore, vulva in females and cloaca in males.

A pair of cuticular expansions in the form of cervical papillae, located at a distance of about 375 µm from the anterior end are present on the lateral sides of the body in both the sexes (Fig 2). The second type of cuticular modification is in the form of longitudinal ridges running antero-posteriorly along the longitudinal axis of the body(Figs. 1, 4 and 5).

The cuticle in general is 7 – 8.5 µm thick, the maximum thickness being at the areas of longitudinal ridges. The ratio of the cuticle to the diameter of the body is 1:32. By Haematoxylin – Eosin staining, the cuticle shows three layers. The darkly staining cortical layer, a lighter and thicker middle layer and a basal layer (Figs. 1, 3 and 21). A faint impression of epicuticle is also observed. But the complex nature of the cuticle is better resolved by histochemical techniques, which reveal that the cortical layer consists of two sub layers: an outer and an inner cortical layer (Figs. 21 and 22). The thickness of the outer cortical layer is 0.5 µm. It is lipo-proteinaceous in nature. The inner cortical layer is positive for carbohydrates and is 1.0 µm thick. Outside to the outer cortical layer lies another entity which stains with Alcian blue and is named epicuticle (Figs. 15 and 16). The light appearing homogenous middle layer stains moderately with lipids, carbohydrates and proteins. It is 3.9 µm thick and the struts originate from this layer. The basal layer is 1.7 µm and passes beneath the struts and the cervical papillae expansion uninterrupted. It is PAS positive and is comparatively less developed (Figs.11 and 12).

Hypodermis: Hypodermis lies between the cuticle and somatic muscle layer and is thickened at four regions to form smaller dorsal and ventral cords and much prominent lateral cords. The cords extend internally and divide the muscle cell layer into four inter-hypodermal groups (Figs. 4 and 5). In a transverse section, the lateral cords measure 57 µm in breadth and 32 µm in thickness. A pore in the form of a thickened bleb like structure connects the hypodermis with cuticle in the lateral cord areas and it appears more of cuticular nature. It looks like a communicating point between the hypodermis and the overlying cuticle. The hypodermal pore is of considerable size and measure 4.1 x 1.5 µm (Figs. 1, 17, 18 and 23).



Figs. 1-8: ***Haemonchus contortus.***

Fig. 1: A diagrammatic representation (reconstructed from longitudinal and transverse sections stained for various histochemical parameters) of body wall showing cuticular ridge (CR), strut (ST), epicuticle (EP), lipoproteinaceous outer cortical layer (OCL), PAS positive inner cortical layer (ICL), median layer (ML) and basal layer (BL) of cuticle, lateral hypodermal cord (LHC), hypodermal pore (HP), lateral excretory canal (LEC), contractile fibrillar zone (FZ) and non-contractile sarcoplasmic zone (SZ) of muscle cell (MC).

Fig. 2: Anterior region showing cervical papilla (CP), mouth (M) and oesophagus (OE). (stained with Methylene blue, mounted in lectophenol).

Fig. 3: T.S. showing outer cortical layer (OCL), inner cortical layer (ICL), median layer (ML) and basal layer (BL) of cuticle, epicuticle (EP), cuticular ridges (CR).

Fig. 4: T.S. showing cuticle (C), cuticular ridges (CR), dorsal hypodermal cord (DHC) lateral hypodermal cord (LHC), ventral hypodermal cord (VHC), muscle cell (MC), Intestine (I).

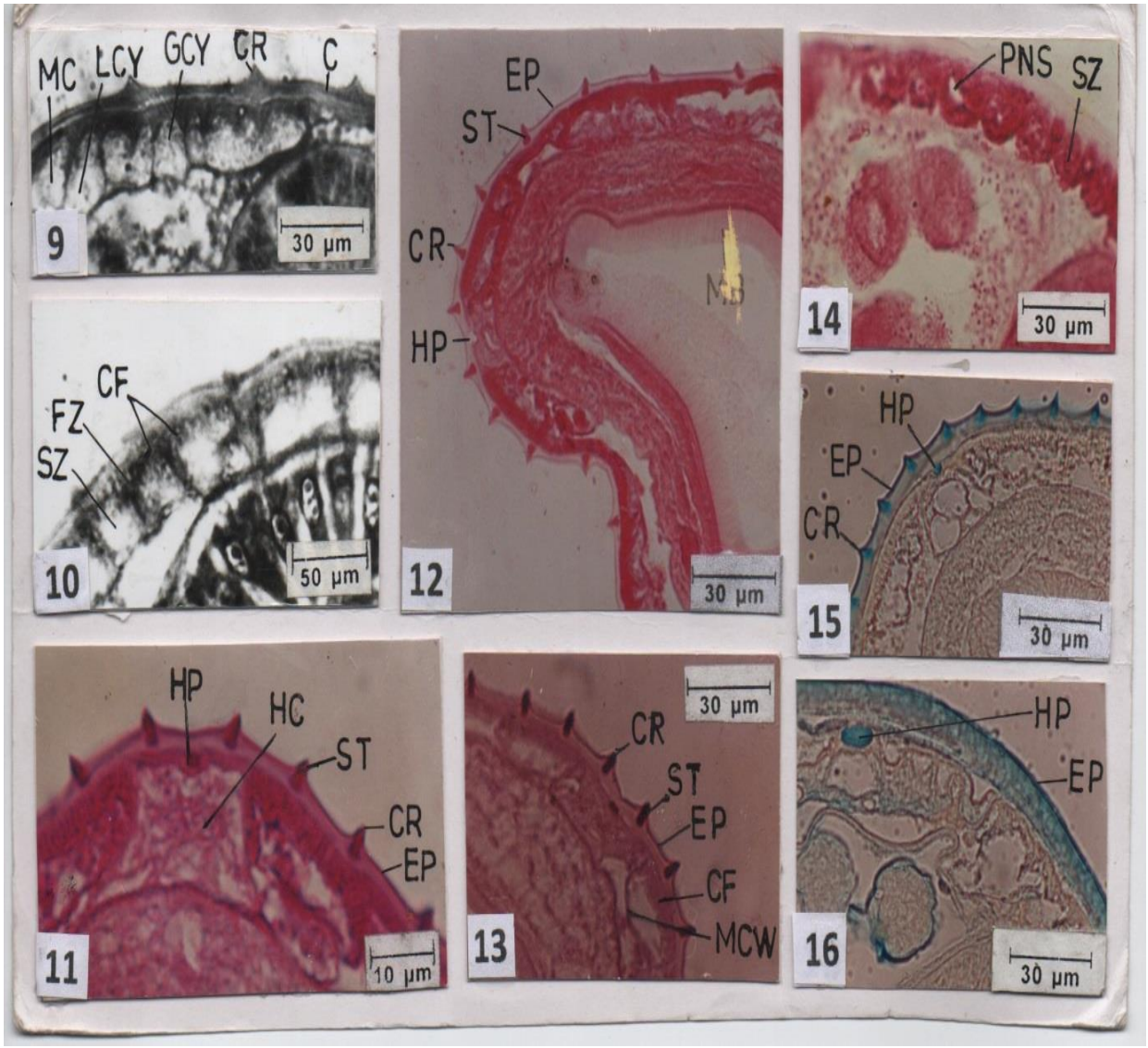
Fig. 5: T.S. of female showing cuticle (C), cuticular ridges (CR), dorsal hypodermal cord (DHC) lateral hypodermal cord (LHC), ventral hypodermal cord (VHC), muscle cell (MC), Intestine (I). ovary (O).

Fig. 6: T. S. from anal region (A) showing body wall (BW).

Fig. 7: T.S. showing lateral hypodermal cord (LHC), lateral excretory canal (LEC).

Fig. 8: T.S. showing oesophagus (OE), nerve ring (NR), lateral nerve cord (LN), ventral nerve cord (VN), muscle cell (MC).

(Fig. 3 to Fig 8, slides stained with Haematoxylin-eosin).



Figs. 9-16: ***Haemonchus contortus.***

Fig. 9: T. S. showing lighter cytoplasm (LCY), granular cytoplasm (GCY), muscle cell (MC) cuticle (C), cuticular ridges (CR). (Haematoxylin-eosin staining).

Fig. 10: T.S. showing contractile fibres (CF), fibrillar zone (FZ) and sarcoplasmic zone (SZ) of muscle cell. (Haematoxylin-eosin staining).

Fig. 11: T.S. showing the distribution of carbohydrates in epicuticle (EP), hypodermal pore (HP), hypodermal cord (HC), cuticular ridge (CR), Strut (ST).
(Periodic acid Schiff staining).

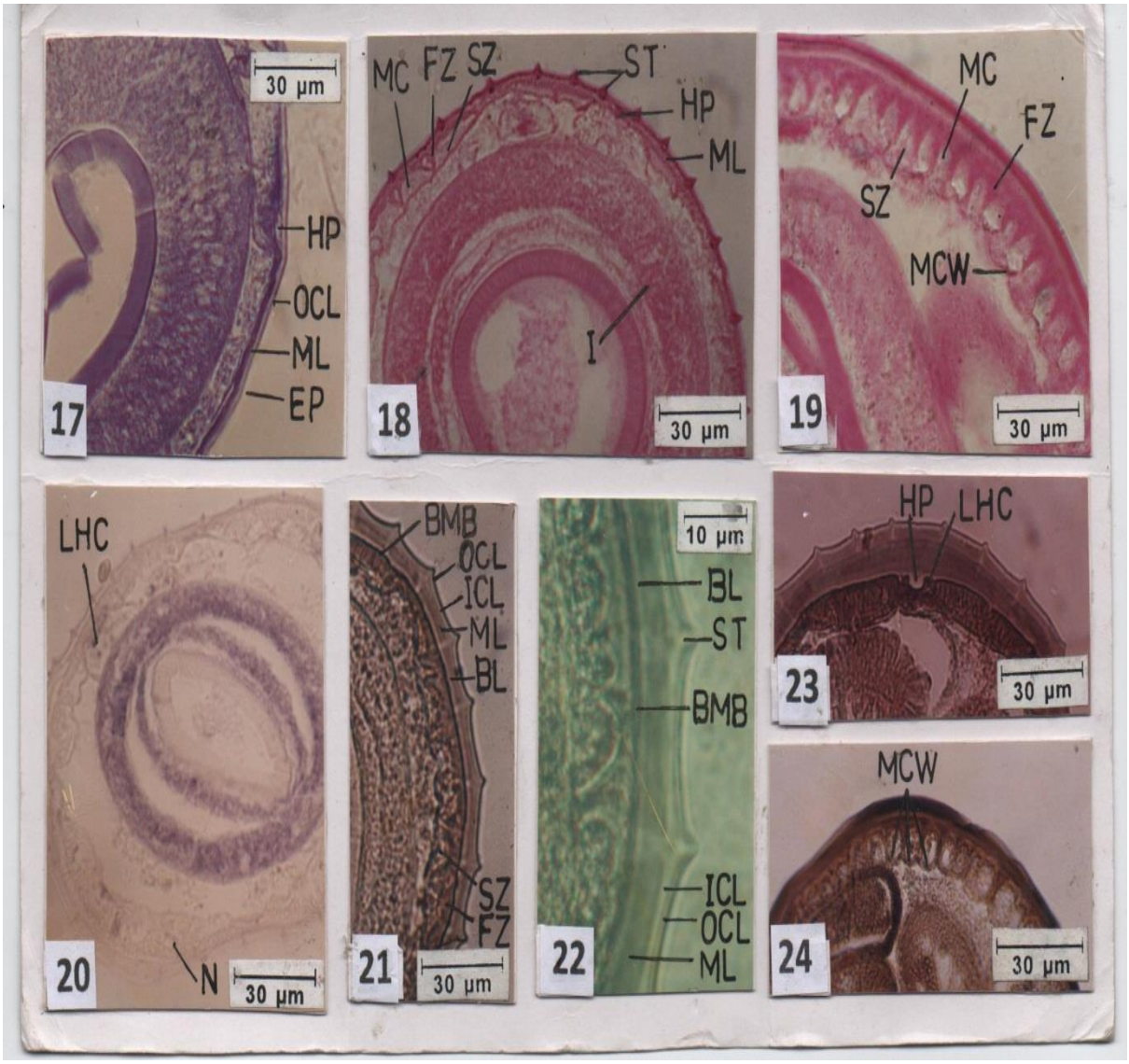
Fig. 12: T.S. showing the distribution of carbohydrates in epicuticle (EP), hypodermal pore (HP), epicuticle (EP), cuticular ridge (CR), microvillar border (MB). (Periodic acid Schiff staining).

Fig. 13: T.S. showing the distribution of glycogen in epicuticle (EP), cuticular ridge (CR), strut (ST), contractile fibres (CF), muscle cell wall (MCW). (Best's carmine staining).

Fig. 14: T.S. showing the distribution of glycogen in sarcoplasmic zone (SZ) and perinuclear space (PNS) of muscle cell. (Best's carmine staining).

Fig. 15: T.S. showing the distribution of acid mucopolysaccharides in epicuticle (EP), hypodermal pore (HP), cuticular ridge (CR). (Alcian Blue staining).

Fig. 16: T.S. showing the distribution of acid mucopolysaccharides of in epicuticle (EP) and hypodermal pore (HP). (Alcian Blue staining).



Figs. 17-24: ***Haemonchus contortus.***

Fig. 17: T.S. showing the distribution of proteins in epicuticle (EP), hypodermal pore (HP), outer cortical layer (OCL). median layer (ML). (Mercuric bromophenol blue staining).

Fig. 18: T.S. showing the distribution of proteins in the struts (ST), Median layer (ML), hypodermal pore (HP), fibrillar zone (FZ) and sarcoplasmic zone (SZ) of muscle cell (MC). (Ninhydrin Schiff's staining).

Fig. 19: T.S. showing the distribution of proteins in muscle cell wall (MCW), fibrillar zone (FZ) and sarcoplasmic zone (SZ) of muscle cell (MC). (Ninhydrin Schiff's staining).

Fig. 20: T.S. showing the distribution of nucleic acids in lateral hypodermal cord (LHC), nuclei (N). (Gallocyanin chromalum staining).

Fig. 21: T.S. showing the distribution of lipids in outer cortical layer (OCL), inner cortical layer (ICL), median layer (ML), basal layer (BL) of cuticle, basement membrane (BMB), fibrillar zone (FZ) and sarcoplasmic zone (SZ) of muscle cell (MC). (Sudan black B staining).

Fig. 22: T.S. showing the distribution of lipids in outer cortical layer (OCL), inner cortical layer (ICL), median layer (ML), basal layer (BL) of cuticle, basement membrane (BMB), strut (ST). (Sudan black B staining).

Fig. 23: T.S. showing the distribution of lipids in lateral hypodermal cord (LHC), hypodermal pore (HP). (Sudan black B staining).

Fig. 24: T.S. showing the distribution of lipids in muscle cell wall (MCW). (Sudan black B staining).

A pair of lateral longitudinal excretory canals is seen running in the substance of lateral hypodermal cords. The lumen of the excretory canal varies from oval to round and is cuticularized. No nuclei are evident in the hypodermal cords (Fig. 7). The nerve ring spans all the four hypodermal cords and the dorsal, ventral and lateral longitudinal nerve cords are seen to lie in the substance of their respective hypodermal cords (Fig. 8).

Muscle layer: Somatic musculature forms a single layer of muscle cells running longitudinally. The hypodermal cords interrupt the continuity of the muscle cell layer dividing it into four intercordal areas (Fig. 4 and 5). The number of muscle cells in the anterior most region is 2 to 3 in each quadrant (or inter hypodermal region) (Fig. 8), whereas in the middle region it increases to 10 or 11 (Figs. 4 and 5) and in the posterior region the musculature is less developed except in the anal and cloacal region (Fig. 6).

The size of the muscle cells in the anterior region of the body is large. It measures 22 µm in thickness with its contractile portion being 9 µm and the noncontractile portion 13 µm thick. The contractile region of the muscle cells is beset with contractile fibers on the basal and lateral sides. The size of the fibers is large in the basal area and gradually diminishes on the sides towards the apical region of the muscle cell (Figs. 8, 9 and 10). The cytoplasm is densely granular towards the basal region and lighter towards the apices of the cells (Fig. 9). Probably the muscle cells are very long as the nuclei are seldom visible. The muscle arms lay closely applied to the muscle layer (Figs. 8 and 9).

Chemical Nature: The epicuticle which also covers the tips of struts, is rich in glycogen, whereas the rest of the cuticle being poor as indicated by Best's carmine staining (Figs. 13 and 14). General carbohydrates are also seen in the epicuticle, the struts, the hypodermal cords, hypodermal pore, muscle cell wall and contractile fibres as revealed by Periodic acid Schiffs technique (Figs. 11 and 12). However, the muscle cells contain high amount of glycogen in their sarcoplasmic region leaving the perinuclear spaces free from it (Fig. 14). The hypodermal pore which is PAS positive, however, is negative for glycogen (Fig. 12).

Acid mucopolysaccharides are distributed over the epicuticle and the struts lying in the longitudinal ridges. Besides, the hypodermal pore communicating with the cuticle too, possesses acid mucopolysaccharides as evidenced by Alcian blue staining (Figs. 15 and 16).

Protein is restricted to outer cortical layer and the median layer only (Fig. 17). Contractile portion of muscle cells and cell walls are highly proteinaceous in nature. Both the outer as well as median layer of cuticle, the struts and hypodermal pore contain proteins with – NH_2 group as revealed by the Ninhydrin Schiff staining (Figs. 18 and 19).

Faint traces of nucleic acids are seen in the lateral hypodermal cords as well as muscle cell nuclei as indicated by Gallocyanin chromalum technique (Fig. 20).

The outer cortical layer is lipoidal in nature. It also extends to the tips of struts beneath the glycogenous layer. The struts themselves are devoid of any lipids. Some lipid is found in the middle layer too, whereas the inner cortical and the basal layers seem to be devoid of it. On the other hand, the basement membrance is intensively lipoidal in nature as indicated by Sudan black B staining (Figs. 21 and 22). The hypodermal areas are also positive for lipids except the hypodermal pores (Fig. 23). The contractile portion of muscle cells and the basal lamina underlying the cuticle possess a considerable quantity of lipid in them. The sarcoplasmic portion of muscle cells show lipid in granular form, whereas the apical walls of muscle cells have lipid as their main constituent (Fig. 24).

DISCUSSION

Harris and Crofton (1957) and Wisse and Daems (1968) have described that the cuticle is a multilayered structure functioning both as a barrier to undesirable elements in their surrounding environment and also as a flexible skeleton.

Haemonchus contortus inhabiting the abomasum portion of stomach of sheep and goat is always exposed to a highly acidic environment with

a pH of approximately 3.0 (Smyth, 1996) and is also subjected to the powerful muscular movements of the stomach which may dislodge it from its niche. It is the ridged, tough and resistant cuticle of the parasite which helps it to survive under such conditions. The distribution, structure and chemical composition of the struts in the cuticle of *Haemonchus contortus* was previously discussed by Singh (2012b).

The cuticle of *H. contortus* is divisible into three main layers i.e., a cortex consisting of external and internal cortical layers, a median and a basal layer. Outside to the external cortical layer is the epicuticle. The structural composition of nematode cuticle varies from two layered in the larva of *Trichinella spiralis* (Beckiett and Boothroyd, 1961) to eight layered in *Oxyuris equi* (Bird, 1958). Two layered cuticles have also been reported in the females of the family Heteroderidae (Wieser, 1953; Ferris and Siegel, 1957; Bird and Rogers, 1965 and Kampfle, 1966). However, in a vast majority of the nematodes a three-layered cuticle is reported. The layers being cortical, median and basal (Lee, 1965; Anya, 1966; Bird, 1971; Cano- Martil *et al*., 2006; Rahemo and Hussain (2009) and Lalchhandama (2010). Some other variations include a four layered cuticle characterizing the infective juveniles of entomopathogenic steinernematid nematodes (Martinez and Souza, 1997) and a 7-8 layered cuticle previously described by Martini (1912) and Martin and Lee (1983) in *Oxyuris equi* and *Nippostrongylus brasiliensis* respectively.

Although there was a generalized concept of cuticle being a three-layered structure as mentioned above. But, Bird (1980) considers that an outer covering is present on the cuticle and has suggested that it is a cell membrance and should be referred as an epicuticle. Since then, the use of terms such as “membrane”, “membrane like”, modified membrane” for different regions of the cuticle have created confusions and implied various concepts regarding its composition and function. Bird (1987) further suggests that the epicuticle is responsible for the secretion of carbohydrate containing glycocalyx. To solve this problem and minimize any further confusion, Wright (1987) has declared that the epicuticle is not a cell membrane, however, with further studies it could be seen as a surface trilaminate differentiation containing lipids. After taking into consideration the different views, Bird and Bird (1991) have concluded that a generalized nematode cuticle consists of an epicuticle, a cortical, a median and a basal layer. Cano-Martil *et al*. (2006) also found this cuticular strata pattern in *Diplotriaena tridens.* The present study on *H. contortus* is also in accordance with this generalized pattern.

In various nematodes, the epicuticle appears to be consisting of glycoproteins and may contain some lipids (Smyth, 1996). In present study on *Haemonchus contortus*, general carbohydrates along with glycogen and acid mucopolysaccharides have been detected in the epicuticle. Earlier, Pease (1966) has described the significance of acid mucopolysaccharides in the nematode cuticle. According to him a selective permeability towards water, gases and certain ions and a barrier towards large molecules of harmful secretions is provided by acid mucopolysaccharides. Dunphy and Webester (1987) have established that the surface epicuticle of the sheath of *Steinernema carpocapsae* is rich in lipids and sugars and may help to produce a semipermeable barrier in regulating cuticle permeability. Thus, it can be concluded that the epicuticle acts as a selective permeability barrier or a resistant layer. Bird and Bird (1991) also suggested that the regulation of cuticle permeability is the chief function of the epicuticle. Similarly, the presence of acid mucopolysaccharides in the epicuticle and the underlying struts of *Haemonchus contortus*, too can be taken as a selectively permeable structural component of the body wall which acts as a barrier towards host enzymes and other harmful substances. Earlier, acid mucopolysaccharides have also been detected on the surface of cuticle in *Pseudoproleptus kherai*, *Diplotriaena tricuspis*, *Oesophagostomum columbianum* and *Toxocara canis* by Sheikher and Garg (1979), Wajihullah and Ansari (1981), Johal (1994) and Page *et al.* (1992).

The main cortical layer is single in most juveniles and many free-living adults, but it is divided into an inner and an outer cortical layer in many parasitic nematodes (Lee and Atkinson, 1976). The external cortical layer of *Ascaris lumbricoides* is a tanned protein (Bird, 1958). In most of the nematodes a proteinaceous outer cortical layer is reported as in *Aspiculuris tetraptera* (Anya, 1966). Similarly, the outer cortical layer of *Paranisakis kherai* and *Setaria cervi* reveal an

adequate number of proteins with – NH_2 and – SH groups and is negative for general carbohydrates and acid mucopolysaccharides (Gupta and Garg, 1976; Gupta and Kalia, 1978). Besides proteins with – NH_2 and – SH groups, a limited amount of acid mucopolysaccharides is also seen in *Pseudoproleptus kherai* as reported by Sheikher and Garg (1979). However, in the present study, the outer cortical layer of *H. contortus* contains a moderate quantity of general as well as – NH_2 bound proteins. In addition, an adequate amount of lipid has also been localized.The lipo-proteinaceous nature of the outer cortical layer was also described by Sood and Kalra (1977). Earlier, the presence of lipid in the outer cortical layer of *Ascaris lumbricoides* has been described by Bird and Deutsch (1957). Lipid along with glycogen in cortex has been detected by Anya (1964) and she has further stated that the cortical lipids were resistant to extraction with pyridine and are therefore bound lipids. The present investigation reveals an adequate amount of acid mucopolysaccharides in the epicuticle and lipid in the outer cortical layer of the cuticle of *H. contortus*. This provides a highly resistant epicuticle-exocortex complex barrier against the chemically unfavourable environment of the host's abomasums. The epicuticle and the outer cortical layer are the only layers which cover the tips of the struts uninterrupted and therefore form a separate entity attributing to the protective function of the cuticle. A similar condition is also reported earlier by Majumdar *et al*. (1996) who have recorded a substantial amount of lipid and hexose containing mucosubstances in the epicuticle-exocortex complex of *Ascaridia galli*. Except for *Setaria cervi* in which a proteinaceous inner cortical layer has been described by Gupta and Kalia (1978) majority of the nematodes possess a glycogenous inner cortical layer. Glycogen has been detected in *Pseudoproleptus kherai* (Gupta and Garg, 1976) and *Oesophagostomum columbianum* (Johal, 1994) in the inner cortical layer. In the present study on *Haemonchus contortus* too, a moderate quantity of carbohydrates is present in this layer.

As compared to the well-defined cortex, the median layer of the cuticle is a relatively structureless layer in most nematodes. Bird and Deutsch (1957) have described the median layer as a homogenous layer with radial striations. However, the middle layer is reported as a transparent structure containing globular electron dense bodies by Beams and Sekhon (1967). Wisse and Daems (1968) described that the median layer in *Rhabditis pellio* is more electron dense than the internal cortical and basal layers, lying on its each side. This layer is uniformly granular and is not traversed by any skeletal structure. In *Trichuris ovis*, Shivali (1991) too, denies the presence of any hard structure in the median layer.

In the present study on *Haemonchus contortus*, the median layer of the cuticle is well developed and the struts extend from this layer to the cortex. It possesses a homogenous matrix which forms a thick layer in the ridged areas and thin in the inter-ridge areas and is interrupted by the presence of struts. Earlier, Lee (1965) has also reported the presence of skeletal struts in the fluid filled median layer of *Nippostrongylus brasiliensis*.

Chemically, the median layer of the nematodes is the most specific. In *Aspiculuris tetraptera*, it contains an adequate amount of carbohydrates and a poor quantity of glycogen and acid mucorpolysaccharides (Anya, 1966). In *Setaria cervi*, (Gupta and Kalia, 1978) the median layer possesses high concentrations of polysaccharides with 1: 2 glycol group together with glycogen. An appreciable quantity of carbohydrates is also reported in the middle layer of cuticle of *Trichuris ovis* (Shivali, 1991). On the other hand, the presence of general as well as – NH_2 bound proteins and lipids have been detected from the matrix layer of *Pseudoproleptus kherai* by Sheikher and Garg (1979). Lipids alongwith glycogen have also been reported in the matrix layer of *Paranisakis kherai* (Gupta and Garg, 1976). Majumdar *et al.* (1996) have determined a moderate amount of bound lipids in the median layer of *Ascaridia galli*. Bird (1971) has reported that the median layer of nematodes is composed of proteins which resemble collagen. The present investigation on *Haemonchus contortus* reveals that the median layer has – NH_2 bound proteins and an appreciable quantity of lipids as its main constituent but the struts present in the median layer are positive for acid mucopolysaccharides, – NH_2 bound proteins and general carbohydrates.

The basal layer of some nematodes is modified to form two or three fiber layers (Lee and Atkinson, 1976). The fibrous nature of the basal layer has

been described in *Oxyuris equi* (Martini, 1912 and Bird, 1958), *Strongylus equinus* (Bird, 1958 and Chitwood and Chitwood, 1950), *Nippostrongylus brasiliensis* (Lee, 1965), *Aspiculuris tetraptera* (Anya, 1966), *Nematodirus battus* (Martin and Lee, 1983), *Strongylus venezulensis* (Martinez and Souza, 1997) and *Ascaridia galli* (Rahemo and Hussain, 2009). The basal layer displays regularly spaced striations in the cuticle of *Necator americanus*, *Ancylostoma duodenale*, *Ancylostoma caninum* and *Trichostrongylus orientalis* (Inatomi *et al*., 1963) The striations in the basal layer of nematodes have a dual function as they act as skeletal structure as well as pore canals (Bird and Deutsch, 1957). Johal (1994) has reported the presence of striations extending from the basal layer to the cortical layer of *Oesophagostomum columbianum* and suggests that these form communication channels between the hypodermis and the cuticle.

On the other hand, the basal layer of *Haemonchus contortus* is neither well developed nor striated. It can be related to the presence of skeletal rods or struts originating from the median layer and extending to the cortical layer which is a characteristic feature of *Haemonchus*. The struts give a skeletal support to the body wall for its locomotory movements and as a consequence the burden of locomotion is shifted from the basal layer to the cortical layer. The cuticle of *H. contortus* has lipids and carbohydrates in more concentrations as compared to proteins. A high quantity of carbohydrates in the form of glycogen and acid mucopolysaccharides is located in the epicuticle, struts and the outer cortical layer. Protein is restricted to the outer cortical and lipid in outer cortical and median layers. Seen under a broad spectrum, in *H. contortus* the epicuticle-cortex complex accounts for the resistant nature of the cuticle, the struts help in locomotion. The median layer contains most of the cuticle essentials like lipids, proteins and carbohydrates which probably are passed to it through the hypodermal pores.

In *H. contortus*, the dorsal and ventral hypodermal cords are smaller in size but the laterals are considerably large and possess a pore which opens at the base of the cuticle thus forming a communication channel between the two. No nuclei are visible in the hypodermal cords. The hypodermis contains a substantial amount of lipids and carbohydrates and faint traces of nucleic acids. The hypodermal pore is positive for carbohydrates, glycogen, acid mucopolysaccharides and – NH2 bound proteins.

Lipids are also detected from the hypodermis of *Setaria cervi*, *Pseudoproleptus kherai*, *Meloidogyne incognita* and *Oesophagostomum columbianum* by Gupta and Kalia (1978), Sheikher and Garg (1979), Marwah and Khera (1987) and Johal (1994). The presence of both fat and glycogen in the lateral hypodermal cords of various nematodes is accounted by Anya (1964) and Savel and Georges (1969) in different nematode species.

According to Lee (1965) and Thompson and Geary (2002) the macromolecules are transported from hypodermis to the cuticle through a system of canals while the micromolecules can pass by diffusion. The presence of appreciable quantity of lipids and carbohydrates in the hypodermal cords of *H. contortus* agrees with the hypothesis that the hypodermis forms the main source of these metabolites which are conveyed to the cuticle through the lateral hypodermal pores to be distributed subsequently to the different layers of the cuticle. The presence of protein along with RNA accounts for the metabolic activity of the hypodermis.

The fibrillar portions of coeomyarian muscles of *H. contortus* extend to sides also, leaving little space for the sarcoplasmic zone. The cytoplasm is more concentrated towards the apical zone of cells which is rich in glycogen. In *H. contortus* the cytoplasmic connections between the somatic muscle cells are not raised into muscle arms but are closely applied to the apical bounding membrane. So, the nerve impulses may not be passing to the far lying muscle cells directly from the nerve cord but from cell to cell. Large concentration of glycogen in the sarcoplasmic zone of muscle cell was observed in *Diplotriaena tricuspis. Oesophagostomum columbianum* and *Litomosoides carinii* by Wajihullah and Ansari (1981), Johal (1994) and Storey and Ogbogu (1991). According to Lee and Atkinson (1976) the glycogen is stored in the muscle cell as a metabolite for energy production. When required or under stress conditions it is converted into simple sugars by the process of glycogenolysis. Glycogen depository of muscle cell is also necessary for providing energy for muscular activity.

In *Tanqua anomala* (Kankal, 1989) as well as *H. contortus* taken for the present study large amounts of glycogen are found in the contractile and the noncontractile sarcoplasmic zones of muscle cell except the perinuclear spaces. As described above, this must be serving a dual purpose, first being used by the muscle cell for its own contractility and secondly as a metabolic reserve. In *H. contortus*, large concentrations of proteins are found in the cell membranes and contractile portion of muscle cells. However, these areas are negative for nucleic acid activity. So, it can be inferred that the protein here is not synthesized; rather it is transported from the basal zone of the intestinal epithelium and is used mainly as a structural component. The presence of protein in the muscle cell has also been reported in *Paranisakis kherai*, *Meloidogyne incognita* and *Oesohagostomum columbianum* by Gupta and Garg (1976), Marwah and Khera (1987) and Johal (1994) respectively.

In present study on *H. contortus*, the contractile portion of muscle cells and the basal lamina underlying the cuticle and apical walls of muscle cells are highly lipoidal. Lipids are also located in granular form in the non-contractile portion of muscle cells. A small quantity of lipids has been reported in the contractile as well as noncontractile parts of muscle cells by Lee (1960) and Johal (1994). However, in present case of *H. contortus*, the stored granular form of lipid in the noncontractile portion probably is being used as a metabolite.

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