RESEARCH ARTICLE

Histochemical and histoenzymatic observations on the intestinal epithelium of *Haemonchus contortus* (Nematoda)

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ABSTRACT

Histochemically, an intense concentration of glycogen, general proteins, lipids, nucleic acids and acid and alkaline phosphatases is seen in the intestinal epithelium of *Haemonchus contortus*. A well developed microvillar border positive for general carbohydrates, -NH_2 bound proteins, lipids, acid phosphatases is present. The microvillar border is totally free from glycogen indicating that the microvilli help in the absorption of simple carbohydrates, which in turn are converted to and stored in the form of glycogen in the intestinal epithelium. The intestine of *H. contortus* does not only act as a place for the transport of absorbed materials but also a tissue of considerable synthetic activity. The presence of proteins and RNA activity indicates that the intestinal protein synthesis pool also distributes a large quantity of protein to the other body organs. A close approximation of intestine with the reproductive organs indicates the trans-membrane flow of nutrients from the former to the later.

Keywords: Intestinal epithelium, microvilli, histochemistry, nematoda, *Haemonchus contortus*.

INTRODUCTION

The nematode *Haemonchus contortus* is a serious pathogenic endoparasite of sheep and other domestic ruminants. 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. 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. Different histochemical parameters have been

described in the intestinal epithelium of various nematodes by Chitwood and Chitwood (1950), Lee (1960), Bird (1971), Jenkins (1970,1973), Johal *et al.* (1997), Johal and Singh (1998), Anderson (2000) and Sood (2006). Previously, the histo-morphological study on the intestinal epithelium of *H. contortus* was performed by Singh and Johal (2004). The present study describes many histochemical variations in the intestine of *H. contortus*, which can fill the hitherto existing gaps in information regarding this aspect. This histochemical localization of various macromolecules will be of significance to understand the metabolic activities and fundamental functional aspects. It can also form the basis in evolving chemotherapeutic measures against this 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 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, lipids, acid phosphatases and alkaline phosphatases 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). For histoenzymatic studies, acid phosphatase paraffin section technique (Ruyter, 1964) and Modified Gomori method for alkaline phosphatese (Fredricsson, 1956) were used. The slides were examined under the microscope and photo micrographed.

RESULTS AND DISCUSSION

The carbohydrate is seen in a diffused form or in a sort of network form in the cytoplasmic region of intestinal epithelium of *Haemonchus contortus.* The intestinal contents and microvillar border also stains pink with periodic acid Schiff's technique indicating the absorption of carbohydrates. The outer covering or basal lamina of intestine contains carbohydrates as one of the main constituent (Fig. 1 and Fig. 2).

A substantial amount of glycogen is aggregated in the intestinal epithelium but the microvillar border is glycogen free (Fig. 3 and Fig. 4). A negligible amount of acid mucopolysaccharides is evidenced at the tips of microvilli (Fig. 8).

In the intestinal region the basal lamina, intestinal epithelium and nuclei reveal a higher concentration of general proteins than the microvillar border. The intestinal contents are also proteinaceous (Fig. 5 and Fig. 6). Proteins with $-NH_2$ group are intensely concentrated in microvillar border and epithelial nuclei than in the cytoplasmic region of the epithelium as evidenced by Ninhydrin Schiff's staining (Fig. 7). In the intestinal region of digestive system, the concentration of proteins in the epithelium is also accompanied by presence of nucleic acids (Fig. 9 and 10). A rich quantity of lipid is seen at the site of terminal web as well as the epithelial cytoplasmic area. Some lipoidal concentration is also observed at the tips of microvilli probably of secretary nature (Fig.11). The rectal glands too are lipoidal in nature (Fig. 12).

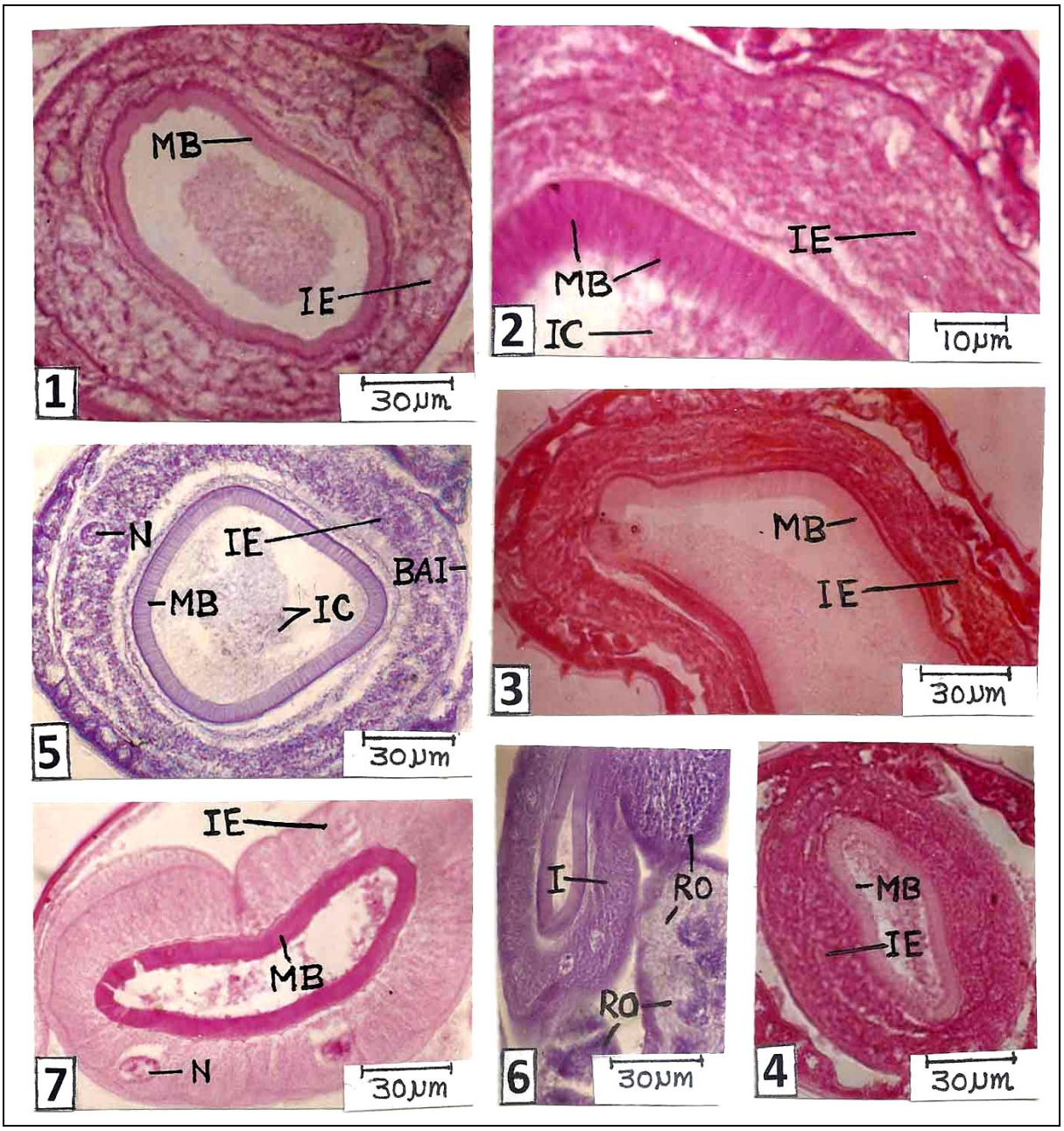

Fig. 1-7: *Haemonchus contortus*

Fig. 1 and 2 : T. S. of intestine showing concentration of general carbohydrates in the intestinal epithelium and microvilli (Periodic acid Schiff's staining); **Fig. 3 and 4 :** T. S. of intestine showing distribution of glycogen (Best's carmine staining); **Fig. 5 :** T. S. of intestine revealing distribution of proteins (Mercuric bromophenol blue staining); **Fig. 6 :** A portion of T. S showing close approximation of intestine and reproductive organs (Mercuric bromophenol blue staining); **Fig. 7:** T. S. of intestine showing distribution of $-NH_2$ proteins (Ninhydrin Schiff's staining).

Abbreviations used: BAI: Basal Lamina of Intestine; I: Intestine; IC: Intestinal Contents; IE: Intestinal Epithelium; MB: Microvillar Border; N: Nucleus; RO: Reproductive Organs.

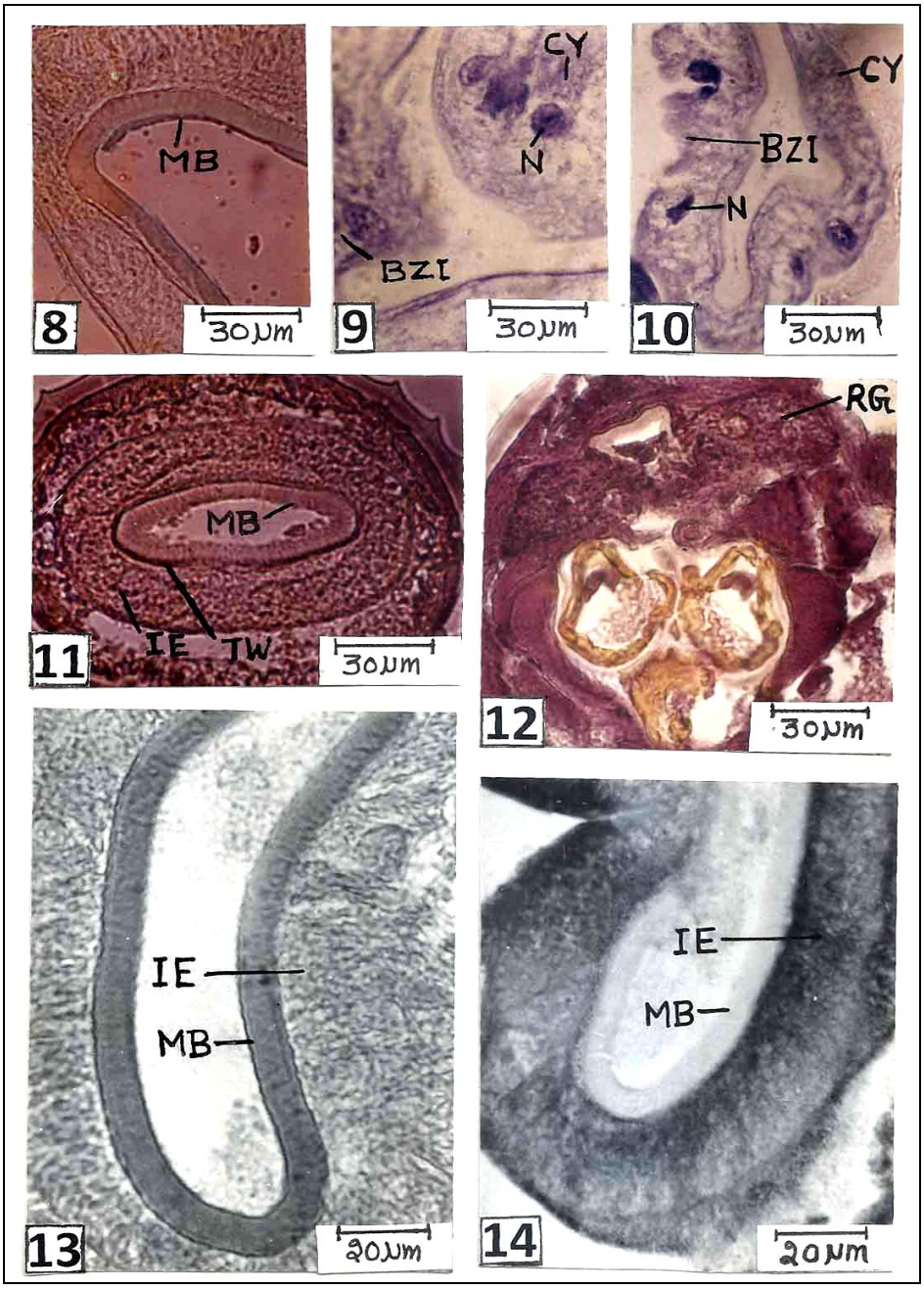

Fig. 8-14 : ***Haemonchus contortus***

Fig. 8: T. S. of intestine showing localization of acid mucopolysaccharides at the tips of microvilli (Alcian blue staining); **Fig. 9 and 10**: A portion of L.S. showing concentration of nucleic acids in the intestine (Gallocyanin chromalum staining); **Fig. 11:** T. S. showing concentration of lipids in the intestine (Sudan black B staining); **Fig. 12:** A portion of T. S. through the cloacal region of male showing distribution of lipids in the rectal glands (Sudan black B staining). **Fig. 13:** A portion of T. S. through the intestine showing presence of acid phosphatase activity in the intestinal epithelium and microvillar border (Acid phosphatase paraffin section technique). **Fig. 14:** A portion of T. S. through the intestine revealing alkaline phosphatase activity in the intestinal epithelium and negative stain for microvillar border (Modified Gomori method).

Abbreviations used: BZI: Basal Zone of Intestinal Epithelium; CY: Cytoplasm; IE: Intestinal Epithelium; MB: Microvillar Border; N: Nucleus; TW: Terminal Web; RG: Rectal glands.

Intense acid phosphatase activity is found in the microvillar border and to some extent in intestinal epithelium (Fig. 13). A substantial amount of alkaline phosphatase is found in the intestinal epithelium, whereas microvillar border is devoid of it (Fig. 14).

Generally, a glycogenous epithelium is the common feature of parasitic nematodes as reported by Von Kemnitz (1912), Enigk (1938) and Von Brand (1938) in different species but the concentration of glycogen content varies depending upon the feeding habit of the various nematodes. In *Haemonchus contortus,* the intestine contains carbohydrates as one of the main constituent. The intestinal epithelium possesses carbohydrates in a diffused form in the cytoplasm and a profuse concentration of glycogen is observed in the intestinal epithelium. Tanaka (1961) while working on *Ascaris lumbricoides* denied the presence of glycogen from the intestinal epithelium. In *Thelastoma bulhoesi,* Lee (1960) described an uneven distribution of glycogen in the intestinal epithelium, being more in the mid region as compared to the anterior and posterior regions. In *Nippostrongylus brasiliensis, Paranisakis kherai, Setaria cervi, Trichinella spiralis* and *Tanqua anomala* an adequate amount of glycogen was observed in the intestinal epithelium by Jamuar (1966), Gupta and Garg (1976), Gupta and Kalia (1978), Takahashi *et al*. (1988) and Kankal (1989) respectively. In the present study on *H. contortus* an intense concentration of glycogen is seen and it is evenly distributed throughout the length of the intestine.

Protein deposits in the form of secretory granules and ribosomes have been reported in the cytoplasm of intestinal cells of *Nippostrongylus brasiliensis* (Jamuar, 1966). In *Paranisakis kherai,* Gupta and Garg (1976) found that the intestinal epithelium contains a moderate quantity of proteins and the chromatin present in the cells is also mercuric bromophenol blue positive. A similar report was given by Gupta and Kalia (1978) for *Setaria cervi.* Proteins bound by both –NH_2 and –SH groups have been detected in the intestinal masses of *Meloidogyne incognita* (Marwah and Khera, 1987). In *Trichuris ovis* and *Oesophagostomum columbianum* appreciable quantities of protein is located in the intestinal epithelium (Johal *et al.* 1997, Johal and Singh 1998). In the present study on *Haemonchus contortus* the intestinal epithelium exhibits a considerable quantity of general proteins as evidenced by Mercuric bromophenol blue technique. In addition, the epithelial nuclei are positive for both general as well as –NH_2 group containing proteins.

Besides these metabolites, some nucleic acid activity is also observed in the intestinal epithelium. Jamuar (1996) has reported that the distribution of RNA in the cytoplasm of intestinal epithelium of *Nippostrongylus brasiliensis* is an indication of protein synthesis at this place. In *Setaria cervi* the basal portion of the intestinal epithelium is rich in RNA content (Gupta and Kalia, 1978). Moderate amount of RNA is also detected in the intestinal epithelium of *Trichuris suis* and *Diplotriaena tricuspis* by Jenkins (1973) and Wajihullah and Ansari (1981) respectively. In *Ancylostoma caninum*, where the presence of proteins is accompanied by RNA activity, Browne *et al.* (1965) have suggested that the cytoplasm of intestinal epithelium acts as a right place for the synthesis of proteins. Von Brand (1952) maintains that the proteins present in the intestinal cells are utilized as a metabolite for the production of energy as well as renewal of protoplasm. In *H. contortus,* the presence of protein and RNA activity indicates that the intestinal protein synthesis pool also distributes a large quantity of protein to the other body organs such as gonads and muscle cells of the body wall, in the latter large amounts of proteins are localized but no nucleic acid activity is evident thus suggesting their dependence for protein on the intestine. It can be inferred that the intestine of *H. contortus* does not only act as a place for transportation of the absorbed material but also a tissue of considerable synthetic activity.

Chitwood and Chitwood (1950) have described that in the intestine of *Cephalobellus papilliger,* the chief constituent of stored food is the lipid. A host of workers such as Lee (1960), Dimitrova (1962), Anya (1964), Jamuar (1966), Reznik (1971), Kankal (1989), Johal *et al.* (1997) and Johal and Singh (1998) have recorded the presence of lipid in the form of granules or fat droplets in the intestinal epithelium of various nematodes. In the present study on *Haemonchus contortus,* appreciable quantities of lipids are found in the intestinal epithelium. The major bulk of lipid seem to be meant for the consumption of reproductive organs lying in close proximity with the intestine, which reveal an enormous quantity of cytoplasmic as well as structural lipid contents. The secretion of some lipoidal enzymes is also indicated in the microvilli. Consequently, *H. contortus* accounts for a tremendous lipoidal activity in it.

The chemical nature of the microvillus border has drawn the attention of many workers for the reason that firstly, like cuticle the microvilli form an interface with the host and secondly, they have a variety of functions. Presence of polysaccharides with 1:2 glycol group and glycogen is reported in the bacillary layer of *Setaria cervi* by Gupta and Kalia (1978). Microvillar border of intestine of *Trichuris ovis* also reveals a tremendous amount of carbohydrate concentration (Johal *et al.*, 1997). In *Haemonchus contortus* too, the microvillar border shows an appreciable quantity of carbohydrates, whereas it is totally free from glycogen. The intestinal contents are also positive for carbohydrates. This indicates that the microvilli helps in the absorption of simple carbohydrates, which in turn are converted to and stored in the form of glycogen in the intestinal epithelium.

The presence of mucopolysaccarides which form a chemical barrier has been reported from the intestinal epithelium and bacillary layer by Gupta and Garg (1976), Wajihullah and Ansari (1981), Marwah and Khera (1987) and Johal and Singh (1998) in *Paranisakis kherai, Diplotriaena tricuspis*, *Meloidogyne incognita* and *Oesophagostomum columbianum* respectively. In present study on *Haemonchus contortus,* very minute quantities of acid mucopolysaccharides are detected at the tips of microvilli. As *Haemonchus* is a blood sucker so its intestine is not exposed to host's gut enzymes.

In *Paranisakis kherai,* Gupta and Garg (1976) reported that the bacillary layer is metabolically rich in general as well as –SH bound proteins. Johal *et al*. (1997) and Johal and Singh (1998) described that the protein forms the main constituent of the microvillar border of the intestine of *Trichuris ovis* and *Oesophagostomum columbianum* respectively. The microvillar border of *H.contortus* too, reveals an intense concentration of $-NH_2$ bound proteins. Considerable amounts of lipids are detected in the microvilli of intestine by a number of previous authors (Gupta and Kalia, 1978; Johal *et al.,* 1997 and Johal and Singh, 1998). In the present study on *H. contortus,* some lipoidal concentration is found at the tips of microvilli which leads to the assumption that some enzyme of lipoidal nature is being released from the microvilli into the lumen of the intestine. A poorly developed terminal web, a cytoplasmic area formed by the fusion of the cores of microvilli at their bases, is observed. The degree of development of terminal web can be related to the kind of fluid which the parasite ingests. Body fluids probably are in a state of assimilation and require less enzymatic activity for their digestion, hence a poorly developed terminal web in *H. contortus.* However a rich quantity of lipid is seen at this site indicating that some enzyme of lipoidal nature is synthesized here.

A highly positive reaction for the activity of acid phosphatase in the intestine of a number of parasitic nematodes is detected by Maki and Yanagisawa (1980a & b). The presence of various hydrolytic enzymes from the intestine of *Trichuris suis, Tetrameres fissispina* and *Haemonchus contortus* is reported by Jenkins (1970), Riley(1973) and Sood and Sehajpal (1978)

respectively. In the present study on *Haemonchus contortus*, both acid as well as alkaline phosphatase activity is seen in the intestinal epithelium. The microvillar border is intensely stained for acid phosphatase activity only, whereas it is negative for alkaline phosphatase.

A characteristic feature of *H. contortus* is that its reproductive organs lie coiled around the intestine and outer wall of the intestine gives an irregular appearance (Singh and Johal, 2004). The uplifting of the basement membrane of the intestine to make a close contact with the reproductive organs indicates the trans-membrane flow of nutrients from the former to the latter. The basal membrane of the intestinal cells probably plays an important role in the rapid uptake kinetics from the intestinal epithelium to the enormous number of developing gametes lying in its vicinity.

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