

Histochemical observations on the oesophagus of *Haemonchus contortus* (Nematoda)

Jatinderpal Singh

Received June 21, 2015 and Accepted September 17, 2015

ABSTRACT : The oesophageal region of digestive system of *Haemonchus contortus* is studied for the histochemical localization of various macromolecules. An intense concentration of glycogen, proteins and lipids is observed in the oesophagus of *H. contortus*. The high concentration of glycogen and lipids in the oesophagus is due to the blood sucking habit of this strongylid nematode.

Key Words: Histochemistry, nematoda, stomodaeum, oesophagus, *Haemonchus contortus*.

Fig. 1 - 4 : *Haemonchus contortus*.

Fig. 1 : T. S. of oesophagus showing concentration of general carbohydrates (Periodic acid Schiff's staining); **Fig. 2 :** T. S. of oesophagus showing concentration of glycogen (Best's carmine staining); **Fig. 3 :** T. S. of oesophagus showing concentration of proteins (Mercuric bromophenol blue staining); **Fig. 4 :** T. S. of oesophagus showing concentration of lipids (Sudan black B staining).

Abbreviations used: BM : Basement Membrane ; BAO : Basal Lamina of Oesophagus; IAR : Interradial Areas ; LB : Luminal Border ; LU : Lumen ; OE : Oesophagus ; OMF : Oesophageal Muscle Fibres.

Haemonchus contortus (Rudolphi) Cobb is a highly pathogenic blood sucking cosmopolitan parasite of sheep and goat. This endoparasitic nematode is of considerable economic importance as it causes severe anaemia, emaciation, weight loss, poor milk yield and wool production. Medium infection causes sheep to lose condition and heavy infection may result into death. The histomorphology of the stomodaeal region of this nematode was studied by Veglia (1915) and Singh and Johal (2001). The present study pertains to the histochemistry of the oesophageal region of stomodaeum of *H. contortus*. The histomorphology together with histochemical study can form the basis for the development of effective chemotherapeutic measures against this serious pathogenic parasite of domestic ruminants.

Materials and Methods

The adult male and female *Haemonchus contortus* extracted from the abomasum of sheep (*Ovis aries*) were washed in 0.85% NaCl solution to remove debris. 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 8µm in transverse and longitudinal planes. The serial sections arranged on slides were stained with various histochemical stains. For the histochemical localization of various macromolecules, 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). For the localization of proteins, Mercuric bromo phenol blue staining (Bonhag, 1955) and Ninhydrin Schiff's staining (Yasuma and Ichikawa, 1953) were used. Acid mucopolysaccharides were studied by Alcian blue (Steedman, 1950) and lipids by Sudan black B staining (McManus, 1946).

The slides were examined under the microscope and photo micrographed.

Results and Discussion

The outer covering or basal lamina of the oesophagus of *Haemonchus contortus* contains carbohydrates as one of the main constituent (Fig.-1). In the oesophageal region the outer covering or basal lamina, the interrarial areas surrounding the triradiate lumen and muscle fibers are rich in glycogen in a descending order of prevalence as indicated by Best's carmine staining (Fig.-2). General proteins have been localized in the bounding membrane, muscle fibres and the lumen of oesophagus of *H. contortus* (Fig.-3). A considerable quantity of lipid in granular form as well as a structural constituent is seen all over the oesophageal tissue of *H. contortus* (Fig.-4).

von Kemnitz (1912), Toryu (1933) and Hirsch and Bretschneider (1937) have reported the presence of glycogen in oesophagus of *Ascaris*, in general. In *Thelastoma bulhoesi* the amount of glycogen present in the oesophageal muscles increases anteroposteriorly being maximum in the isthmus (Lee, 1960). Anya (1964) has determined the presence of glycogen in the muscle fibres of oesophagus of some oxyurids such as *Aspicularis tetraptera*, *Enterobius vermicularis* and *Syphacia obvelata*. In the former although glycogen is observed in the form of granules throughout the tissue of corpus portion of oesophagus. However Gupta and Kalia (1978) reported some amount of glycogen from the supporting membrane of glandular oesophagus of *Setaria cervi*. In *Trichinella spiralis*, the stichocytes reveal massive aggregates of glycogen as reported by Takahashi *et al.* (1988).

Histochemical studies on strongyle form of oesophagus of *H. contortus* revealed that the bounding membrane of oesophagus contains appreciable quantity of carbohydrates. Glycogen is distributed over the basal lamina, interrarial areas surrounding the trira-

diate lumen and muscle fibres.

In *H. contortus*, the oesophageal tissue is also rich in lipids in granular as well as structural form. Lee (1960) has also reported the presence of small amount of fat lying between the muscles in the oesophagus of *Thelastoma bulhoesi*. Gupta and Kalia (1978) reported the presence of large amount of phospholipids in the glandular oesophagus of *Setaria cervi*. The presence of phospholipids in the stichocytes and hypodermal base of bacillary band has been observed in *Trichuris suis* by Jenkins (1970). However in *Aspiculuris tetraptera* and *Oesophagostomum columbianum* the oesophageal tissue is reported to be totally free from lipids (Anyia, 1964; Johal, 1988).

The above observation revealed that in oxyurids glycogen is concentrated in the bulbous part of the oesophagus and in filarid type of oesophagus it is only seen in the supporting membrane. Whereas in trichuroid type and strongyle forms of oesophagus the distribution of glycogen is the maximum throughout

the oesophageal tissue. In *Haemonchus contortus* the presence of glycogen is also accompanied by lipids. It may be due to the powerful sucking activity of the strongyle form of oesophagus. Singh and Johal (2001) reported that double set of marginal fibres in oesophagus of *Haemonchus contortus* contribute in expanding and increasing the capacity of the lumen of oesophagus. Since *Haemonchus* is a blood sucker, so the blood can pass rapidly down to the intestine during feeding, through the much expanded oesophageal lumen.

Acknowledgements

The author is thankful to Prof. (Dr.) Manjit Johal for her guidance, encouragement and constant support for this research work. The author is also indebted to the Professor and Head, Department of Zoology, Punjabi University, Patiala and Principal, Baring Union Christian College, Batala for providing necessary facilities to carry out the research.

References

- Anyia, A.O., 1964. The distribution of lipids and glycogen in some female oxyuroids. *Parasitology*, **54** : 555-566.
- Best, F., 1906. *Z. Wiss. Mirk.* **23** : 319. Cited from: *Histochemistry, Theoretical and Applied*. A.G.E., Pearse (Ed.), J. Churchill, London (1968).
- Bonhag, P.F., 1955. *J. Morphol.*, **96** : 381. Cited from: *The Structure of Nematodes*, A. F. Bird (1971), Academic Press, New York & London.
- Gupta, N.K. and Kalia, D.C., 1978. Histochemical studies on *Setaria cervi*, a filarid nematode of veterinary importance. *Rivista Di Parassit.*, **37**(2-3) : 265-275.
- Hirsch, G.C. and Bretschneider, L.H., 1937. Der Intraplasmasatichstoffwechsel in den Darmzellen von *Ascaris lumbricoides*. *Protoplasma*, **29** : 9-30.
- Jenkins, T., 1970. A morphological and histochemical study of *Trichuris suis* (Schrunk, 1788) with special reference to the host parasite relationship. *Parasitology*, **61** : 357-374.
- Johal, M., 1988. Investigations on histoarchitectural, biochemical and histochemical aspects of some organ-systems of *Oesophagostomum columbianum*, Curtice, 1890. *Ph.D. Thesis, Punjabi University, Patiala (India)*.
- Lee, D.L., 1960. The distribution of glycogen and fat in