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Histochemical Localization of Carbohydrates in Schistosoma spindale

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Abstract: *In all living cells, the carbohydrates are the central pathway for the supply of energy needed for metabolic reactions. Trematodes utilise carbohydrates as the primary source of energy at the parasitic stage. Several factors influence the content of carbohydrates of trematode, Schistosoma spindale such as oxygen tension of the environment, sexual differences, seasonal changes, diurnal variation, nutritional state of the host and stages of the life cycle. Histochemical localization of carbohydrates is carried out in the present work to investigate the importance of carbohydrates in the metabolism of S.spindale. The muscular regions of the suckers happens to play an important role in the adaptation of the fluke in the blood. The present work displays significant and crucial aspect of fluke's life by the histochemical demonstration of tissue carbohydrates. The studies related to the present work essentially reveal vital aspects of the ability of S. spindale to adapt itself in the host Bubalus bubalis.*

Key Words: *Schistosoma spindale, trematodes, histochemical techniques , Periodic Acid Schiff's Method.*

1. INTRODUCTION:

Carbohydrates are polyhydroxylic aldehydes or ketones comprising one of the major groups of naturally occurring organic material. They are classified as monosaccarides, oligosaccharides and polysaccharides. They are widely distributed in animal tissues as glycogen which is the most common reserve formed of these compounds. In animals, carbohydrates vary in quality and quantity and they perform three important functions. Firstly, they form an important part of structural component. Secondly, they are stored as major energy reserves. Thirdly, they are crucial to energy metabolism. In all living cells, the carbohydrates are the central pathway for the supply of energy needed for two metabolic reactions anabolism and catabolism. In the parasite, these carbohydrates are either catabolised for release of energy or polymerized and stored as reserve polysaccharides. To demonstrate the enzymatic study of carbohydrates in the tissues of helminths, histochemistry is the best proven option. It involves techniques that provide biochemical and molecular information about the structure and function of cells, tissues and organs. This histochemical work tends to display the reactions clearly as it deals with the identification of chemical components in cells and tissues. The given research work have been undertaken to localize histochemically the carbohydrates in the sections of the trematode, *S. spindale*.

2. LITERATURE REVIEW:

Endo-parasitic worms exhibits a pronounced carbohydrates metabolism and contain large quantities of carbohydrates essentially in the form of glycogen derived from the host. All endo-parasitic helminths exhibit a dependence upon anaerobic carbohydrate metabolism to obtain energy regardless of the amount of environmental oxygen available. Parasitic helminths inhabiting anaerobic or micro-aerophilic environments contain large reserve of endogenous polysaccharides in the form of glycogen. They obtain their energy almost exclusively through the fermentation of carbohydrates as they are the best sources of anerobic energy than either proteins or fats. It has been known for more than 100 years (Bernand, 1859; Foster, 1865) that parasitic worms contain polysaccharides. As early as Bernand (1859), Von Brand(1973) demonstrated the presence of carbohydrates in helminths while Foster(1865) made conclusive quantitative and qualitative observations in these helminth worms. Weinland(1901, 1902) presented the initial papers on carbohydrate metabolism in helminths. The glycogen found in the parasite helminths does not appear to differ in any significant degree from the found in vertebrates (Symth, 1966). Orrell el al.(1966) demonstrated that the broad spectrum of molecular weight of glycogen though present in *Hymenolepis diminuta* varied with the method of glycogen extraction used. Experiments conducted by Roberts et al.(1972) on *H. diminuta* showed that glucose incorporation was higher in high molecular weight dextrin, than in low molecular weight dextrin. Barrett(1981)suggested that low and high molecular weight forms of glycogen in helminths have different molecular weight and different turnover rates. Both high and low molecular weight fractions of glycogen, decreased during starvation in *Fasciola hepatica* and *H. diminuta* and both factors increased rapidly again on feeding. Glycogen constitute nearly 10-20% of dry weight of parasitic helminths. Digeneans store 2% to 30% of dry weight as glycogen. The larval

cestodes show higher and more consistent amount of glycogen than the corresponding adults. Nematodes and acanthocephalans also contain relatively large amounts of stored glycogen varying between 10% to 60% of their dry weight (Barrett, 1981). Histochemical aspects studied in schistosomes have unravelled to a wide extent the localization of major metabolites and enzymes of schistosomes. Histochemical demonstration of beta-gluconidase was localized in the schistosomes by Fripp,1966. Characterization of the carbohydrates of *S. japonicum* adult worm, egg and cercaria by analysis of lectin binding and antibody reaction (Beisler et al.,1984). Localization and identification of *S. mansoni* /KLH-crossreactive Components in Infected Mice was done by Cecilia and Ewert(2003). Carbohydrates detection in the hepatic egg-Granuloma system using Lectin Histochemistry was carried out by Melo Junior et al. (2009). This study aimed to evaluate egg-granuloma system in hepatic tissues in experimental Schistosomiasis using lectin histochemistry. Proteins in *S.spindale* was histochemically localized by Vanita et al.(2018). Histochemical localization of Glutamate dehydrogenase activity in *S. spindale* was done by Vanita et al.(2019). However, there has been lacuna observed in the histochemical studies related to carbohydrates in *S.spindale.* The present work is a fragment to explore this area of research in *S.spindale.*

3. MATERIALS AND METHODS:

Histochemical Studies:

Histochemical demonstration of metabolities in *S.spindale* was done by the paraffin embedding process of the parasite to make blocks which are the sectioned and stained. Periodic Acid Schiff's (PAS) method were used for the staining histochemically.

Periodic Acid Schiff's (PAS) Method (McManus, 1948)

Deparaffinised sections were brought to water through descending grades of alcohol. Oxidised for 10 minutes in 1% periodic acid. Washed in distilled water. Immersed in Schiff's reagent for 15 minutes. Washed in distilled water. Immersed in Schiff's reagent for 15 minutes. Washed in tap water. Dehydrated through ascending grades of alcohol. Cleared in xylene. Mounted in DPX mountant. Carbohydrates were stained magenta.

4. RESULT:

The histochemically localized sections depicted the Carbohydrates stained magenta in colour by Periodic Acid Schiff's (PAS) technique in *S. spindale*. Some of the significant results are as follows:

- The tegument showed considerable activity of the carbohydrates(Fig. 1,2,3,4,5,6,7,8,9). The body wall showed significant amounts of carbohydrates.(Fig. 1,2,3).The PAS positive parenchyma which is made up network of cells and fibres enclosing irregular spaces is seen in Fig.1,2, 3.
- The different sections of the female Schistosoma spindale are stained substantially with PAS(Fig.4,5). The magenta stained section reveal caecum of the intestine of female with remnants of blood in the caecum(Fig. 5).
- The section of the coiled male Schistosoma spindale showed substantial deposition of carbohydrates.(Fig.6, 7, 8,10)
- The characteristeric feature of Schistosoma, being a blood fluke and female residing in the gynaecophoric canal of the male, is evident by the stained sections of the Schistosomes found in Fig. 9,11,12,13,14,15.
- There is high staining of PAS observed in the sucker. The rim of the pedunculated sucker showed intense staining indicating the immense quantities of glycogen reserve which is evidently utilized for the functioning of these muscles.(Fig.16,17).
- The testes is conspiciously seen showing the PAS positive region in the male Schistosoma spindale.(Fig. 18,19).

5. DISCUSSION:

Carbohydrates are the primary energy source which trematodes are capable of utilising at the parasitic stage. This is evidenced by a high level of glycogen reported from the species of parasitic helminths. Glycogen as a reserve carbohydrate is non-diffusible and exerts low osmotic pressure. The glycogen deposits of parasites represent as the energy reserve. Anaerobic metabolism necessitates a steady supply of glycogen which can be stored by the parasites and hydrolyzed to glucose whenever energy production is required. The low yield of energy under anaerobic fermentation in endoparasites necessitates in high storage of endogenous glycogen(Smyth, 1969). Helminths in general and trematodes in particular store high quantities of glycogen. This glycogen is derived from the host dietary carbohydrates which are absorbed by the parasites in the form of smaller sugar molecules. The intense positive reaction to PAS by *S. spindale* in the present study suggested that tegument contained abundant carbohydrate reserve. Similar studies were reported by Erasmus and Ohman(1963), Pantelouris(1964), Ohman(1965) in trematodes. Besides, the regions of the bodywall are having a thick musculature which corresponds to the fact that there is release of energy from the carbohydrate reserve stored in the bodywall to facilitate extensive movements in the blood. However, the

gynaecophoric canal has been the area where the tissue carbohydrates are found to be extensive to counteract to the ability to hold the female and have an active role in the motility of the worm. The testes contained intense amount of carbohydrates. The supporting cells of the testes are cellular with irregular, multinucleate epithelial cells which are believed to be nutritive in function. Von brand(1939), Bullock(1949) Crompton(1963,1965) had reported similar observations of the localization of glycogen in the testes. Tieliens et al(1989) have reported large quantities of glycogen from adult *S. mansoni*. Female *S. spindale* was found to contain an abundance of distribution of carbohydrates in the present study. As the worm lives in a medium(blood) which guarantees a continuous supply of glucose from glycolysis, glycogen is not having solely a structural function. A large part of glycogen is rapidly degraded mainly to lactate. The present study correlates to the researchers who have carried out extensive work on the histochemical localization of carbohydrates in the tissues of trematodes and cestodes. Noteworthy among them are the works of Prenant(1922), Yoshimura and Yokogawa(1958), Reddy(1982), Sabeha(1994), Mrunalini(1988), Parvathi(2007),Vanita(2007).

6. CONCLUSION:

The blood parasite *S. spindale* lives in an atmosphere having low oxygen tension. As aerobic respiration is not possible in such an environment, the parasite derives the energy required for its catabolic activities by anaerobic fermentation of carbohydrates. Hence, Carbohydrates forms the primary source which the parasite is capable of utilising in its microaerophilic environment. The survivability of *S. spindale* within the blood of the host depends upon its ability to accumulate sugars when they are abundant in its macro-environment and to resist the loss of sugars to the blood when the carbohydrate levels in blood are low. In the present study, the histochemical study emphasises on the role of glycogen as an important polysaccharide reserve in the parasite which is metabolized for energy production as well as for the different metabolic processes of to adapt itself in the host.

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Annexure: Figures

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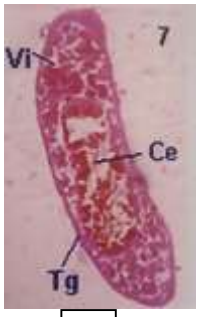

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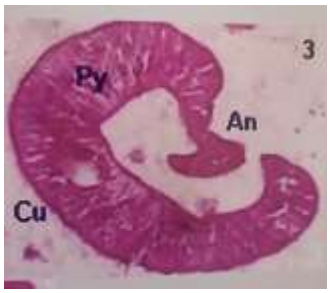

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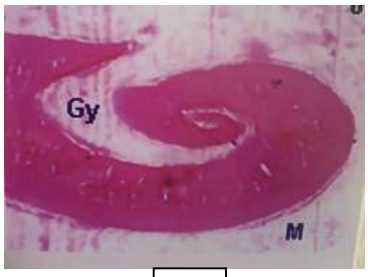

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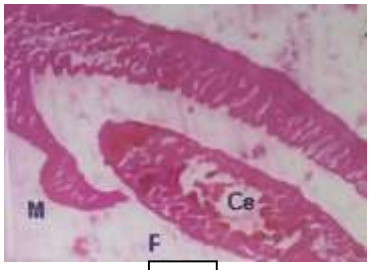

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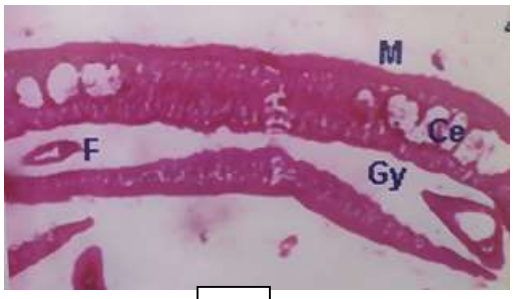

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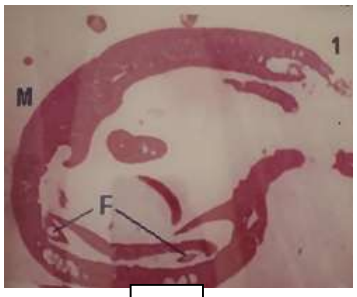

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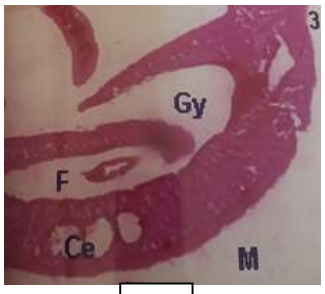

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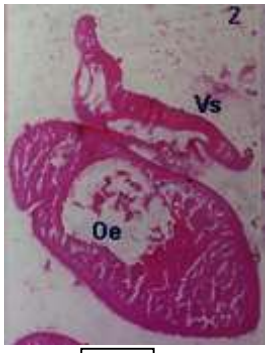

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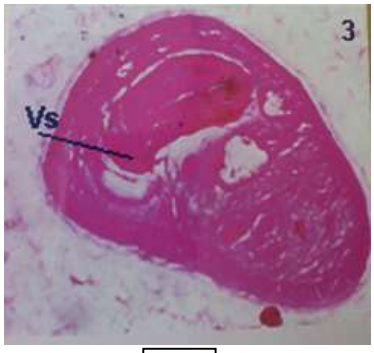

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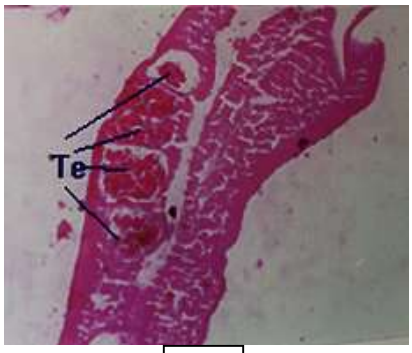

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Fig. 1,2,3. Section of the bodywall of male and female Schistosoma spindale showing mucosubstances stained regions. Fig.4,5 Mucosubstances found to stained substantially in the transverse sections of the female. Fig.6,7,8,10. Section of the different male Schistosoma spindale showing mucosubstances stained significantly. Fig. 9. Section of male and female stained regions to show the presence of mucosubstances. Fig. 11,12,13, 14,15. Different sections of female in the gynaecophoric canal of the male Schitosoma which show the mucosubstances stained regions in magenta colour. Fig.16,17. The mucosubstances stained different sections of ventral sucker. Fig.18,19.Mucosubstances stained very conspiciously in the testes of the male Schistosoma spindale.