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HISTOCHEMICAL LOCALIZATION OF LIPIDS IN SCHISTOSOMA SPINDALE

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Abstract: *Lipids are organic compounds occuring in all animal tissues. The most important role of the lipids is to provide fuel for metabolism.. They also play a crucial role in the enzyme regulation, cell surface recognition, glycoprotein sythesis, membrane transport and also in the expression of surface antigenic determinants. In combination with proteins as lipo-proteins , they form important constituent of all the naturally occuring cell membranes. They are found in tissues as glycolipids. The lipids concentrations of various tissues of an animal is highly dependent on the carbohydrate and proteins fractions since all the three substrates are interconvertible in their intermediary metabolism, the dietary status of the host and various other factors. Histochemical localization of lipids has been carried out in the present work using the Sudan Black 'B' technique to investigate in Schistosoma spindale, a trematode parasite of Bubalus bubalis. The present study displays the importance of lipids in the ability of S. spindale to a life in the ruminant host.*

Key Words: *Schistosoma spindale, Phospholipids, Histochemical techniques ,Sudan Black 'B' Technique.*

1. INTRODUCTION:

Lipids are heterogeneous compounds related to fatty acids. They occur in all living tissues having a common property of insolubility in water but soluble in non-polar inorganic solvents. The importance of lipids as one of the fundamental classes of biological compounds has been well established. Lipids occur in small fractions in all animal cells and tissues. The lipoproteins contribute to the structural integrity of the cell organelle being important constituent of the biological membranes like cell-membrane, endoplasmic reticulum, mitochondria, golgi complex and nuclear membrane. Lipids in animals, serve as efficient source of energy, yielding per gram over twice as many calories as do the carbohydrates or proteins(White et al., 1973). They also provide mechanical support to the organism serving as an insulating material in subcutaneous tissues and around other certain organs against the loss of heat. Lipids have a variety of cellular function. They play an important role in enzyme regulation, cell surface recognition, glycoproteins synthesis, membrane transport and also in the expression of surface antigenic determinants. Lipids are found to be associated with proteins or carbohydrates as lipoproteins or glycolipids(Smyth, 1969). The most important role of the lipids from the quantitative point of view is that of providing fuel for metabolism. Histochemical localization of lipids has been carried out in many cestodes, nematodes and trematodes (both in adult and larval forms) but not much work has been done in schistosomes. Histochemical studies depend primarily on stains such as Sudan Black 'B' and Nile Blue which are used to demonstrate the distribution of lipids in the cells or tissues.. Histochemical techniques are of immense importance to demonstrates the parasite as a whole or its organs or tissues or cells are too small to yield substantial quantity for chemical analysis. The present work of histochemical localizations of the lipids in *Schistosoma spindale* is an attempt to signify the role of lipids in the adaptation of the parasite in the host, *Bubalus bubalis.*

2. LITERATURE REVIEW:

An enormous research work in the understanding of lipids in helminths has been done since few decades. There are significant studies in nematodes and trematodes while similar studies in few species of schistosomes are very scanty. Beach et al.(1973), Fripp et al.(1976) in *S. mansoni* and Coles(1975), Chappell(!980) in *Fasciola hepatica* demonstrated and showed that trematodes are capable of absorbing host fatty acids and incorporating them into all components of lipids. Di Conza and Basch(1976) observed accumulation of lipids in *S. mansoni in vitro*. Mc Manus et al. (1975) demonstrated that the sporocysts of *Microphallus similis* utilized under aerobic conditions ^{14}C glucose, galactose, fructose and maltose and incorporated the label into lipid and other compounds. Mc Manus and Smyth (1978), stated that lipid content of the parasite varies with the type of species and also with different strains of the same species. Mills et al.(1981) identified glycolipids apart from phospholipids in *Taenia crassiceps*. Hrzenjak et al. (1984) isolated glycolipid complex from *F. hepatica*. Histochemical lipid studies on *S. mansoni* adults maintained *in situ* and *in vitro* was carried out by Haseeb et al (1984). Histochemical and thin-layer chromatographic analyses of neutral lipids in *S. japonicum* adults and their worm-free incubates was carried out by Haseeb et al.(1986). Furlong (1991) reported that parasitic helminths modify fatty acid chain lengths and synthesize fatty acid metabolities. Histochemical localization of

lipids, secondary metabolities and lignin in healthy and *Meloidogyne incognita*, infected Okra (Abelmoschus esculentus(L.) Moench) was carried out by Dubey and Trivedi(2012). Histochemical localization of proteins in *S.spindale* was done by Vanita et al.(2018). Comparative histochemical study of four enzyme activities on some organs of male *S. mansoni* and *S. haematobium* was done by El Shabasy et al.(2018). Vanita et al.(2019) had histochemically demonstrated MDH in *S.spindale*. To fill the lacuna related to the study of the lipids in *S.spindale*, the present work is an insight into the histochemical demonstration of lipids in the sections of *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. Sudan Black 'B' method were used for the staining lipids histochemically.

Sudan Black 'B'Method (Lison and Dagnelie,1935)

Sections were washed in distilled water. Sections were then placed in 60 % triethyl phosphate for 30secondss. The sections were stained in Sudan Black B at 20 ºC for 10 minutes. These are washed in triethly phosphate for 30 seconds. These are then, washed in distilled water and mounted in glycerine jelly. Lipids material including phospholipids are stained black.

4. RESULTS:

Histochemical localizations of lipid content in the sections of S. *spindale* revealed the following results:

- Histochemical observation in the tissues of *S. spindale* evidenced moderate to intense deposits of lipids stained by Sudan Black 'B' method. The lipid regions of the sections of *S.spindale* were seen as black deposits with Sudan Black 'B' technique.
- The tegument showed heavy deposits of lipids as evident by the regions of sections stained black by Sudan Black 'B' technique(Fig.1,2,3,4,5,6,7,8,9,10,11). Considerable amounts of lipids was localized in the parenchyma(Fig. 1,2,3,5,6). The muscular regions of the body wall showed substantial lipids(Fig. 5,6).
- The localization of lipids was seen in the sections of male *S.spindale* coiled around the female(Fig. 1,2,3,4,7,9). The section of male *S.spindale* is seen in the fig. 8,10,19 showed the distribution of lipids.
- The transverse sections of the female showing distribution of lipids was seen in Fig. 22,23. The longitudinal sections showing regions of the caecum stained with Sudan Black 'B' was very evidently seen in the female *S.spindale*(Fig.13,17).
- The sections reveal female in the gynaecophoric regions of the male showing the localization of lipids(Fig. 14,18,20).
- The gynaecophoric canal showed profuse deposits of lipids.(Fig. 18,20). The longitudinal sections of the male and the female *S.spindale* showing distribution of lipids can be seen in Fig. 15,16.
- The ventral sucker of the male *S.spindale* showed intense deposits of lipids.(Fig. 21)
- The reproductive regions of the male and the female showed moderate to intense deposits of lipids in the sections (Fig. 12, 17). 3 to 4 testes is found in the section of the male *S. spindale*. Thick blackish granules were found in the testes(Fig.12). An intense distribution of lipids was found in the ovary(Fig.17).

5. DISCUSSION:

Lipids serve as a source of high energy and play a major role in maintaining structural integrity and resistance to surrounding environment during carbohydrate starvation. The results of the present study give a fair picture of the distribution of lipids and their role in the adaptation of *S.spindale*. The tegument showed intense deposits in the present study, which can be accounted by the fact that lipids contribute to the energy reserve when there is extensive contraction in response to the movement of the parasite in the current of the host's blood. Mills et al.(1984) proposed that the parasites utilize glycolipids to evade the host immune response. Schistosomes coat themselves with host antigens usually glycolipids to escape detection by the host.(Smithers et al. 1969; Dean 1974). Thompson et al. 1960 and many other workers were of the opinion that the presence of lipids in the tegument suggested that lipids may be assisting in offering resistance to combat the stress and strain imposed by the environment. Intense lipid activity in the tegument could be analysed in the light of the role played by the bodywall in the transport of lipophilic substances. The role of phospholipids is insulating a series of enzymes in an organized lipoprotein matrix in the mitochondria which are responsible for the electron transfer and oxidative phosphorylation. This fraction of lipids may also play a role in cation transport and permeability at various interfaces due to its anionic property (Smyth,1966). Similar function attributed

to the significant amounts of lipids in the tegument of *S.spindale* can be inferred by the author in the present study. The significance of lipids in parenchyma of trematodes and therefore *S.spindale* in the present study, may be suggestive of the mechanical role it plays in the structure and support of the internal organs. It also assists in surface transport. Barrett(1981) and Gupta et al.(1974) suggested that lipids act as structural components necessary for providing mechanical support to various organs of the parasite. Intense distribution of lipids has been observed in the reproductive organs in the present investigation. In the present study, lipid deposition in the vitellaria can be inferred as there is cope-up strategy of high egg production by parasite. It is well known that vitellaria function not only in providing the nutrients to the developing zygote but also in the formation of the egg shell. The energy needed for such constant activity is furnished by phospholipids and lipoproteins. So also lipids are also essential component in male reproductive tissue of *S.spindale*.. Although significant amount of lipids have been reported in the intestinal caeca of few species of digenetic trematodes, the only postulation advanced so far relative to its function is the study of Morris (1968) who suggested that the droplets which he observed with the electron microscope might be by-products of host's heaemoglobin degradation. In the present study, lipids droplets were observed histochemically in the caecal epithelia and lumina of *S.spindale*. It can be suggested that lipid droplets occurring in the lumina of the intestinal caeca are excretory by-products, excreted through the gut as in the case of metacercaria of *Leucochloridium constantiae* (Harris and Cheng,1973). Significant activity of lipids was found in the male and the female *S.spindale*. The gynaecophoric canal which holds the female is found to have profuse amounts of lipids. Barrett(1981) reported that parasitic nematodes, trematodes and cestodes can synthesize their own complex phospholipids by pathways as found in mammals. Histochemical studies carried out by the author substantiates role of lipids in *S. spindale*. The results of the present investigation, have helped us to confer that lipids are biologically significant as structural compounds necessary for providing mechanical support to the organs of the parasite, growth factors, providing resistance to the external factors acting on the parasite, cell membrane components in the form of lipo-protein components of the cuticle and other cell membranes transport agents in transport mechanism.

6. CONCLUSION:

Lipids are of immense importance due to their involvement in cellular structures. Lipids are the main dietary sources of energy along with carbohydrates Lipids can be formed in the body from carbohydrate, protein or lipid precursors. Some essential unsaturated fatty acid cannot be formed by the animal body. It has been suggested by helminthologists that many synthetic lipid pathways requiring energy expenditure were probably abandoned by parasitic helminths during evolution because the absorption of these lipids which are readily available in the host's blood. The present investigation has been carried out to histochemically to demonstrate lipids in the sections of *S.spindale*. Significant and useful information of the present study validates the role of lipids in the adaptation of the fluke to the sanguivorous life. The various alterations of both the substrates and enzymes visualized in the present investigation open new vistas for further qualitative and quantitative research.

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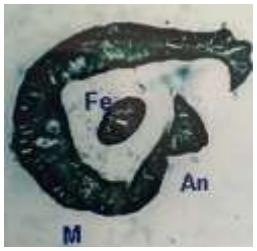
Annexure: Figures



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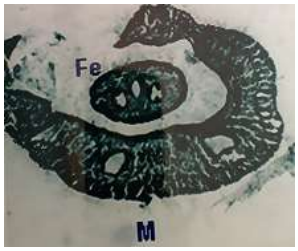

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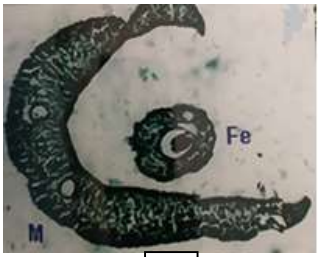

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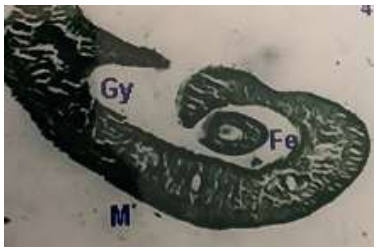

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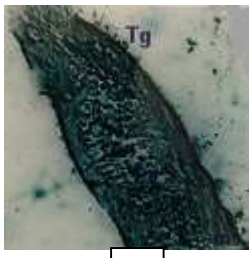

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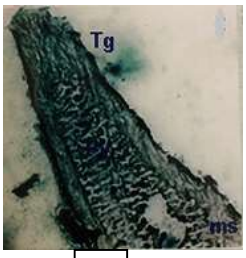

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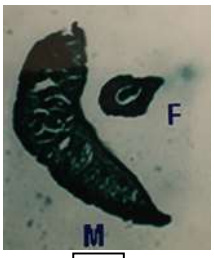

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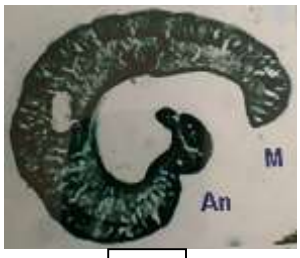

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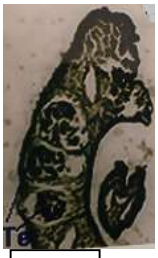

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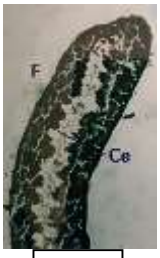

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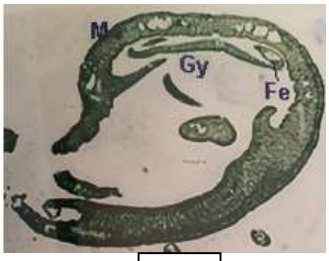

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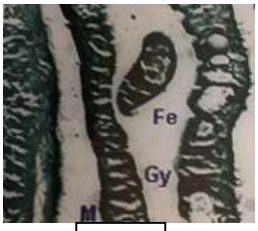

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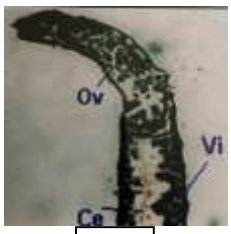

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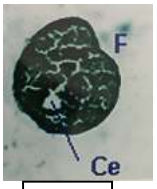

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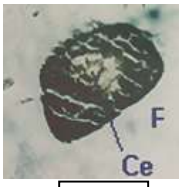

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Fig. 1,2,3,4,7,9. Male *S.spindale* coiled around the female showing localization of lipids. Fig. 5,6. Body wall of *S.spindale* showing localization of lipids.. Fig.12. Male *S.spindale* showing testes stained regions of lipids. Fig. 13. Localization of lipids in the Female *S.spindale* showing ceacum. Fig.17. Female *S.spindale* showing caecum and ovary localized with lipids. Fig. 8,10,19. Male *S.spindale* showing localization of lipids. Fig. 21. Male S.*spindale* showing Ventral Sucker showing lipids stained regions. Fig. 22,23. Transverse section of female S. *spindale* showing localization of lipids. Fig. 11,14,18,20. Sections of female in the gynaecophoric canal of the male showing lipids stained regions. Fig. 15,16. Longitudinal sections of female in the gynaecophoric canal of the male *S.spindale* showing localization of lipids.