HISTOCHEMISTRY

INTRODUCTION

In spite of the undoubted and unquestioned importance of trematodes as a group of harmful helminth parasites, parasitologists are no longer confining themselves to purely taxonomic studies but are also interested in knowing their physiology and internal functioning. For this purpose they are employing modern methods of investigations relating to histochemical, biochemical and histopathological studies.

Each of these methods has its own importance, but in order to reach correct conclusions it sometimes becomes necessary to use these methods in combination.

Many workers have made contributions to the histochemistry of trematode parasites. Prominent amongst those who have contributed to the knowledge of chemical constituents in trematodes are: Axmann (1947); Yamao (1952); Monne (1959); Burton (1960, 1962); Lal and Srivastava (1960); Bjorkman et al. (1964); Ohman (1965, 1966a, 1966b); Bogitosh (1968); Smith and Brooks (1969); Subramaniam (1970); Reader (1971); Yarygina (1972); Gupta and Sharma (1973, 1974); Song (1974); Fried and Butler (1977); Vijylakshmi (1980); Venkata Ramakrishna (1981) and Smirov (1982).

Keeping in view the significant role played by the tegument in this group of parasites in providing defensive
mechanism against the action of various hydrolytic enzymes constituting the ecological niche of these parasites, stress has been laid to work out the chemical nature of this organ.

The ultrastructure of the tegument of *Fasciola hepatica* has been investigated by Threadgold (1963) and Bjorkman and Thorsell (1963). The tegument consists of a syncytial surface layer, which is connected by tube-like protoplasmic processes to individual nucleated areas of cytoplasm, the tegumental cells, lying in the parenchyma. There are two types of tegumental cells: the type I cell has numerous dense mitochondria and a dense, spherical secretory product, whereas the type 2 cell has less dense mitochondria and numerous biconcave, disc-shaped secretory bodies.

**Observations**

**Proteins**

The tegument in both species *Allocreadium dollfusi* and *Helostomatis derog* stained intense blue in the mercury bromophenol blue test showing the presence of general proteins (Fig. 1, 2).

For the demonstration of SH and SS groups ferric ferricyanide and performic acid Schiff techniques were employed.
The tegument in both species revealed the presence of SH groups after ferric ferricyanide technique. In *A. dollfusi* reaction is uniform throughout the tegument as it stained intense blue uniformly, but in *H. dero* the reaction is not uniform throughout (Pmg. 3, 4).

In performic acid schiff for SS groups the tegument in both the species studied reacted negatively showing the absence of SS groups (Pmg. 5, 6).

The absence of NH₂ groups in the tegument of both species is shown by a negative ninhydrin schiff reaction (Pmg. 7, 8).

**Carbohydrates**

Moderate PAS reaction has been observed in the tegument of *A. dollfusi* (Pmg. 9), whereas the tegument in *H. dero* stained intensely in PAS technique (Pmg. 10). PAS positive reaction is given by a variety of substances including polysaccharides, glycoproteins, glycolipids, unsaturated lipids and phospholipids.

With Best's carmine stain the tegument in *A. dollfusi* stained lightly (Pmg. 11). In *H. dero* the tegument gives negative reaction to this stain (Pmg. 12). The light stain in the tegument is not due to the presence of glycogen. This is due to the presence of glycoprotein, complexes.
The tegument in *A. dollfusi* gives intense positive reaction in the Alcian blue technique (Pmg. 13). Moderate reaction to the Alcian blue test has been observed in the tegument of *H. dero* (Pmg. 14). There is distinct under rim of acid mucopolysaccharides staining intensely with Alcian blue technique. The matrix of the tegument in *A. dollfusi* stains intensely (Pmg. 13). But in *H. dero* feeble reaction is given by matrix of the tegument (Pmg. 14).

Toluidine blue was used to study metachromasia. The tegument in both the species of these digenetic trematodes studied show β-metachromasia of varying intensity (Pmg. 15, 16).

**Nucleic acids**

For the study of DNA and RNA Feulgen's technique and methyl green pyronin were employed.

The tegument in both the species stain light to moderate pink in methyl green pyronin stain (Pmg. 17, 18). In *A. dollfusi* the tegument shows the presence of pyroninophil granules (Pmg. 17). It is concluded after trichloroacetic acid extraction for RNA; the pink coloration is not because of the presence of RNA, and the pyronin G of the mixture is not acting as an elective stain for RNA but only as a simple basic dye.

The complete absence of DNA from the tegument of both the species studied has been conclusively proved by negative results in Feulgen's technique (Pmg. 19, 20).
DNA, polysaccharides and proteins

With Himes' and Moriber's triple stain the tegument in both the species pink up red colour for polysaccharides, yellow for proteins. No DNA is observable in the tegument of both species (Pmg. 21, 22).

Lipids

The tegument in *A. dollfusi* stains homogenously and intensely with Sudan black B (Pmg. 23), whereas that of *H. deroê* is moderately stained with light and dark patches (Pmg. 24). These observations show the general presence of lipids in the tegument of digenetic trematodes and that the amount of lipids varies from species to species. In order to find out the nature of these lipids other tests such as Nile blue and acid haematin techniques were applied. In nile blue sulphate the tegument in *A. dollfusi* stains intensely (Pmg. 25), whereas in *H. deroê* this stain imparts light colouration (Pmg. 26). This test shows that the lipids of the tegument are predominantly acidic in nature.

In Baker's acid haematin test the tegument in both species react positively (Pmg. 27, 28) providing the presence of phospholipids.

TEGUMENTAL CELLS

Tegumental cells are clearly observed in mercury bromophenol blue and methyl green pyronin G tests (Pmg. 1, 18).
In methyl green pyronin G the nuclei are stained characteristically green with methyl green of the mixture which is elective stain for DNA and cytoplasmic granules red with pyronin G of the mixture which is elective stain for RNA. In mercury bromophenol blue test the nuclei as well as the cytoplasm of the tegumental cells are stained blue.
Parenchyma

Recently Smith and Halton (1983) worked out the detailed structure of parenchyma. According to them much of the available space between the organ systems in the trematode body is occupied by cells of the parenchyma and fibers of interstitial material. For from being a simple packing tissue, the parenchyma is considered to be a complex system of cells engaged in carbohydrate metabolism and transport. In Fasciola, the cells are large and polymorphic and usually contain substantial amount of α- and β-glycogen. Glycogen stores in the parenchymal cell are sequestered within autophagosomes, derived from membrane synthesized by the cellular mitochondria and then hydrolysed by lysosomal enzymes produced by the GER. Golgi system. The process results not only in the release of glucose but, with the formation of secondary lysosomes and residual bodies, essential constituents molecules are recycled and conserved within the cells.

Parenchymal cells are structurally related to one another and to the tegument, gut, muscles and excretory and reproductive systems by means of gap junctions. These junctional complexes are seen as sites of inter-cellular communication which facilitate the transport of substances throughout the body in the absence of an obvious circulatory system.
Fibrous interstitial is a connective tissue of collagen-like fibres in a granular, carbohydrate matrix which remifies between the parenchymal cells and around organ systems. It appears to be skeletal in function and together with the fibrous basal lamina of the tegument and gastrodermis, provides anchorage for the muscle fibres and against which they can exert a force during contraction. The muscles are attached to this fibrous skeleton by dermosome like plaques or dense bodies.

OBSERVATIONS

Proteins

In mercury bromophenol test for proteins the parenchymal fibres in *A. dollfusi* and *H. derop* are stained distinct blue (Pmg. 1, 2). Proteinaceous material is present in parenchymal spaces in both the species studied. Parenchymal cells are clearly observed in this test. However, their number varies from species to species. In *A. dollfusi* they are found more in number (Pmg. 1), whereas in *H. derop* their number was less (Pmg. 2).

With ferric ferricyanide stain in *A. dollfusi* and *H. derop* parenchymal fibres stained intensely and no proteinaceous material has been observed in parenchymal spaces (Pmg. 3, 4).
In performic acid schiff techniques the parenchymal fibres in both the species studied stain light pink (Pmg. 5, 6). Granules have been seen in parenchymal fibres.

With ninhydrin schiff for NH₂ group, parenchyma in both the species studied stain moderately (Pmsg. 7, 8).

Carbohydrates

The parenchymal fibres in both the species studied stained intensely in PAS technique. The intensity of stain is more in *A. dollfusi* than in *H. deroë* (Pmg. 9, 10). The fibres are coarse in both the species because of granulation on their surface (Pmg. 9, 10). These fibres enclose definite spaces which also contain PAS positive granules. The parenchymal fibres are moderately stained in *H. deroë*, especially in the middle region of the body.

In Best's carmine stain for glycogen in *A. dollfusi* the parenchymal fibres are intensely stained. Deposition of glycogen in the form of granules and their aggregates along the parenchymal fibres are clearly observed in this species (Pmg. 11). In *H. deroë* diffuse staining throughout is seen after Best's carmine stain (Pmg. 12).

In Alcian blue technique the parenchymal fibres in *A. dollfusi* is distinctly stained (Pmg. 13). The parenchymal spaces do not contain any acid mucopolysaccharide granules. In *H. deroë* the parenchymal fibres are lightly stained (Pmg. 14).

The parenchymal fibres in both the species studied stain metachromatically with toludine blue stain (Pmg. 15, 16).
**Nucleic acids**

The nuclei of parenchymal cells are Feulgen positive (Pmg. 17,18). In methyl green pyronin G test, nucleus stains blue for DNA, whereas the nucleolus picks up red colour for RNA (Pmg. 19,20).

**Combined test for DNA, polysaccharides and proteins**

With Himes and Moriber stain the nuclei of the parenchymal cells in both the species studied pick up green stain for DNA (Pmg. 21,22), the cytoplasm of the cell is red because of the presence of proteins, polysaccharides and parenchymal fibres stain yellow for protein.

**Lipids**

In Sudan black B the parenchymal fibres are stained at places and are completely unstained at other places (Pmg. 23,24). The stain is also observed aggregated in lumps at the junction of parenchymal fibres enclosing spaces. This is not due to the presence of lipids, only fat coloureant dye is seen aggregated in big lumps. The parenchymal spaces don't contain any SBB positive material.

With Nile blue stain the lipids of parenchyma of both species stained indicating the presence of acidic lipids (Pmg. 25,26).

In acid haematin test the parenchymal fibres in both species stained blue showing the presence of phospholipids in parenchyma (Pmg. 27, 29).
DISCUSSION

TEGUMENT

Proteins:

The tegument in both species is proteinaceous as indicated by positive results in mercury bromophenol blue and this observation supports those made by Erasmus and Ohman, 1963; Ohman, 1965, 1966a and 1966b. According to Lal and Srivastava, 1960 observations on Fasciola hepatica tegument of digenetic trematode is proteinaceous in nature.

Performic acid-Schiff reaction do not demonstrate clearly the presence of -SS group in both species evidenced by negative reaction with this stain. This observation is in full accord with those observations of Lal & Srivastava and Pearse, 1968. Lal and Srivastava found S-S groups to be absent from cuticle in Fasciola hepatica.

Carbohydrates:

Studies by Axmann (1947); Yamao (1952), Monne(1959), Von Brand and Mercado (1961), Burton (1962) and Muller (1966), have given us an idea of the general distribution of glycogen in digenetic trematodes. Our observations on the site of glycogen storage in tegument are in general agreement with those of the earlier workers in that we have found glycogen to be absent from the tegument of a number of digenetic trematodes studied by them.

The PAS method is widely used for the detection of glycogen-like polysaccharides, but is also known to give a
positive reaction with collagen, reticulin and similar proteins, and furthermore with certain mucopolysaccharides. The superficial strip of the tegument stains because it contains acid mucopolysaccharides (Monne, 1961). The red staining of tissues other than the above was taken to mean the presence there of glycogen. The distribution emerging is in agreement with that derived by Ortner-Schonbach by a different method. Again Bjorkman et al. (1963) interpreted the PAS positive granules in the tegument of _Fasciola hepatica_ as glycogen. In conclusion, our observations are in accordance with Monne (1959) and not with those of Bjorkman et al. (1963) who on the basis of the Schiff reaction alone concluded that glycogen was present in tegument of _Fasciola hepatica_.

The tegument of both species stained intensely in tests for acid mucopolysaccharides. Their granules were located in the matrix of tegument. In addition to it acid-mucopolysaccharides were also found on the external surface of tegument. These observations of the author are in accord with the observations of Threadgold, 1968, 1976, Morris and Threadgold, 1968 on _Schistosoma mansoni_ and Erasmus, 1967 on _Cyathocotyle bushiensis_.

**Nucleic acids**

The complete absence of DNA and RNA from the tegument of both species has been conclusively proved by negative results in Feulgen's technique. This observation is in conformity with that those of Bjorkman and Thorsell (1964) who reported the absence of DNA & RNA in the cuticle of _Fasciola hepatica_.

In pyronin-methyl green stain, light to moderately pink staining material has been observed in tegument. This is due to the presence of pyroninophil granules in tegument which cannot be considered as RNA. A negative result with pyronin-methyl green in the tegument of *Schistosoma mansoni* obtained by Wilson and Barnes (1974). These observations are further supported by the observations of Erasmus and Ohman, 1963, 1965, 1966a and 1966b) on some strigeoid trematodes.

**Lipids:**

Abundant lipid droplets in the tegument of both species were reported after Sudan-black B staining. Threadgold (1968) found similar condition in *Haplometra cylindracea*. According to Lal & Srivastava (1960) lipids are absent from the tegument of *Fasciola hepatica*. In the present investigations Sudanphilia observed in the tegument of both species.

Phospholipids were present in tegument as it stained distinctly in Baker's acid haematin and the lipid fraction in the tegument is predominately composed of phospholipids. These observations are similar to Burton (1960) who reported phospholipid in the distal cytoplasm of *Haematoloechus medioplexus*.

In Nile blue sulphate the tegument of both species stains blue, indicating that the lipids of the tegument are predominantly acidic in nature.
The histochemistry of the cyst wall of Fibricola indicus n.sp.

There have been few studies on the nature and histochemistry of trematode metacercarial cysts. The lack of a comparative study of this aspect of trematode morphology prompted this investigation. In the present investigations the nature of the cyst wall is examined histochemically.

Previously, investigations have also been made to determine the chemical composition of cyst wall by Herber (1950); Singh & Lewert (1965); Lenhoff, Schroeder and Leigh (1960), Lynch and Bogitsh (1962) and Dixon (1965) and Dixon and Mercer (1967).

**OBSERVATIONS**

The cyst wall of *Fibricola indicus* consists of two main layers. More recently Dixon (1965) has described two layers in the outer cyst wall of *Fasciola hepatica* and two layers in the inner cyst wall one of which is further subdivided into three.

**Observations**

**Outer cyst wall:** The gland cells concerned with the formation of the outer cyst wall are the dorsal and ventral. So the histochemical reactions of the dorsal and ventral region of the wall are slightly different as they are secreted in part by different gland cells.
Proteins

The outer and inner layer of outer cyst wall reacts negatively with mercury bromophenol blue stain only middle layer react positively showing the presence of general proteins (Pmg. 29).

With ferric ferricyanide stain outer cyst wall stained intensely blue. But its layers were not found to be differentiated showing the presence of SH groups (Pmg. 30).

Ninhydrin-Schiff imparts no colouration to outer cyst wall (outer, inner, middle) suggesting the absence of -NH2 group (Pmg. 31).

Carbohydrates

With periodic acid schiff stain cyst wall stained intensely showing the presence of polysaccharides (Pmg.32).

With toluidine blue outer cyst wall stained moderately on dorsal region whereas it stained weakly on ventral region (Pmg. 33) showing the presence of β-metachronasia.

With best carmine stain the outer cyst wall reacts negatively showing the absence of glycogen (Pmg. 34).

With Alcian blue stain the cyst wall reacts positively showing the presence of mucopolysaccharides (Pmg. 35).
**Nucleic acids**

With methyl-green pyronin outer cyst wall reacts negatively (Pmg. 36).

**Lipids**

With Sudan Black B the cyst wall stained moderately showing the presence of lipids (Pmg. 37).

With Nihe blue and acid haematin (Pmg. 38). It has been observed that lipids are acidic in nature and phospholipids are present.

**Inner cyst Wall**

Two layers can be distinguished dorsally and laterally and both show the same histochemical reactions.

**Proteins**

With bromophenol blue the inner cyst wall stained intensely showing the presence of general proteins (Pmg. 29).

With ferric ferricyanide stain the inner cyst wall stained intensely blue showing the presence of -SH groups (Pmg. 30).

With ninhydrin Schiff stain the wall gave negative reaction showing the absence of NH$_2$ groups (Pmg. 31).
Carbohydrates

With periodic acid schiff the wall stained distinctly red showing the presence of polysaccharides (Fig. 32).

With toluidine blue inner cyst wall stained lightly showing the presence of β-metachromasia (Fig. 33).

With Best's carmine stain the inner cyst wall reacts negatively showing absence of glycogen (Fig. 34).

With Alcian blue stain the inner cyst wall stained distinctly blue (Fig. 35) showing the presence of mucopolysaccharides.

Lipids

With Sudan black B the inner cyst wall stained intensely black showing the presence of lipoproteins (Fig. 38).

With Nile blue and acid haematin stain the wall stained moderately showing the presence of acidic lipids and phospholipids (Fig. 39, 37).
Herber (1950) described briefly the composition of the cyst wall enclosing the metacercaria of Notocotylus urbanensis and concluded that it was proteinaceous in nature. In the present species studied all the layers reacted in varying degrees to the PAS and bromophenol blue test showing that all the layers were mucopolyproteinous in nature. These observations support those made by Singh and Lewert (1959).

The metacercarial cyst wall of Fasciola hepatica has been investigated by Dixon (1965). The wall has been shown to consist of four major layers. In Fibricola indicus the first or outermost layer which is incomplete ventrally is composed of tanned protein and probably provides the major protection for the metacercaria. This outer layer bears on its inner surface a thin fibrous layer of mucoprotein and acid mucopolysaccharides. The third layer is not uniform in comparison but contains on outer region of mucoprotein, a median acid-mucopolysaccharide region and inner zone of neutral mucopolysaccharide. The fourth and innermost layer consists of lamellae of protein stabilized by disulphide linkages held in a protein-lipid matrix. This layer is incomplete ventrally, where there occurs a thickened
mucopolysaccharides plug. The fourth layer again composed of protein and it provides considerable protection to the metacercaria. These observations are in conformity with those of Dixon (1965) and Dixon and Mercer (1967).
CONCLUSIONS

1. Glycogen has been found to be absent from the tegument of both the species of the digenetic trematode studied.

2. The tegument of both the species is proteinaceous; containing no S-S groups.

3. Amount of bound carbohydrates is variable in the parenchymal spaces of different species.

4. No nuclear material could be found in the tegument of both the species.

5. Lipids are present in the tegument and these are transported to this site by parenchymal fibres which contain lipids in one portion that only stained with Sudan black B whereas the portion having no lipids remained unstained.

6. Acid mucopolysaccharides granules are found throughout the parenchyma in both the species.

7. Parenchymal fibres are proteinaceous.

8. Parenchymal spaces of digenetic trematodes studied contain glycogen and neutral polysaccharides.

9. Outer cyst wall of Fibricola indicus consists of acid mucopolysaccharides, neutral mucopolysaccharides, protein, lipoprotein and glycoprotein.

10. Inner cyst wall, mucoprotein, acid mucopolysaccharide, neutral mucopolysaccharide, keratinized protein in protein and lipid matrix have been observed.
Pmg-1. S.S. of *Ailocreadium doflfusi* showing the presence of general proteins in the tegument (T) and parenchymal fibres (PF). Tegumental cells (TC) in close proximity to the tegument are also seen. The parenchyma is seen to be composed of darkly stained parenchymal fibre (PF) and parenchymal cells (PC).

Zenker/Mercury bromophenol blue.

Pmg-2 S.S. of *Helostomatid as doro* showing the presence of general proteins in the tegument (T) and parenchymal fibres (PF).

Zenker/Mercury bromophenol blue.
Pmg-3  T.S. of *Allocreadium dollfusi* showing the concentration of substances containing SH groups in the tegument (T) and parenchyma (P).

    Zenker/Ferric ferricyanide.

Pmg-4  S.S. of *Helostomatis deroe* showing the presence of material containing SH groups in the tegument (T) and parenchyma (P).

    Zenker/Ferric-ferricyanide.
Pmg-5. T.S. of *Allocreadium dollfusi* showing the absence of S.S. groups in the tegument (T) and parenchyma (P).

Zenker/Performic acid Schiff.

Pmg-6  S.S. of *Helostomatid aereoe* showing the absence of S.S. groups in the tegument (T) and parenchyma (P).

Zenker/Performic acid Schiff.
Pmg-9  S.S. of *Allocreadium Bollfusi* showing the presence of polysaccharides in the tegument (T) and parenchymal fibre (PP).

Zenker/PAS

Pmg-10  T.S. of *Helostomatis deroe* showing the presence of polysaccharides in the tegument (T) and parenchymal fibre (PP), lumps of Schiff positive material are seen in parenchymal space (PS).

Zenker/PAS.
Pmg-11 T.S. of Allocradium dollfusi showing the absence of glycogen in the tegument (T) and presence of glycogen in the forms of granules along the parenchymal fibre (PF).

Bouin's/Best's carmine

Pmg-12 S.S. of Helostomatis deroe showing the absence of glycogen in the tegument (T) and presence of glycogen in the form of granules along the parenchymal fibre (PF).

Bouin's/Best's carmine
Pmg-13 T.S. of *Allocreadium dollfusi* showing the presence of acid mucopolysaccharides in the tegument (T) and parenchyma (P).

Bouin's/Alcian blue

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Pmg-14 T.S. of *Helcostomatis deroe* showing the presence of acid mucopolysaccharides in the tegument (T) and parenchyma (P)

Bouin's/Alcian blue.
**Pmg-15** T.S. of *Allocreadium dollfusi* showing the presence of $\beta$-metachromasia in the tegument (T) and $\beta, \gamma$ both in the parenchyma (P).

Bouin's/Toluidine blue.

**Pmg-16** S.S. of *Helostomatis deroe* showing presence of $\beta$-metachromasia in the tegument (T) and $\beta, \gamma$ both in the parenchyma (P).

Bouin's/Toluidine blue.
Fig. 17  S.S. of *Helostomatis deroe* showing the absence of RNA in the tegument (T) and parenchyma (P).

Zenker/Methyl green pyronin

Fig. 18  T.S. of *Allocreadium dollfusi* showing the absence of RNA in the tegument (T). Parenchymal cells are seen with distinctly stained nuclus (N) showing the presence of DNA.

Zenker/Methyl green pyronin
Fig-19 S.S. of Helostomatids deren showing the absence of DNA from the tegument (T).

Zenker/Feulgen

Fig-20 S.S. of Allocreadium dollfusi showing the absence of DNA from the tegument (T).

Zenker/Feulgen
Pmg-21 T.S. of Allocreadium dollfusi showing the presence of proteins and carbohydrates in the tegument and absence of DNA from the tegument (T).

Zenker/Himes and Moriber

Pmg-22 S.S. of Helostomatia deroe showing the presence of proteins and carbohydrates in the tegument and absence of DNA from the tegument (T).

Zenker/Himes and Moriber
Pmg-23 S.S. of *Allocreadium dollfusi* showing the presence of lipids in the tegument (T) and parenchymal fibres (PF).

F-Ca/PC/Sudan black B.

Pmg-24 S.S. of *Helostomatis deroe* showing the presence of lipids in the tegument (T) and parenchyma (P).

F-Ca/PC/Sudan black B.
Pmg-25 S.S. of *Allocreadium dollfusi* showing the acidic nature of lipids present in the tegument (T) and parenchymal fibres (PF).

F-Ca/PC/Nile blue

Pmg-26 S.S. of *Helostomates deroe* showing the acidic nature of lipids present in the tegument (T) and parenchymal fibres (PF).

F-Ca/PC/Nile blue.
Pmg_27 S.S. of *Allcreadium dollfusi* showing the presence of phospholipids in the tegument (T) and parenchymal fibres (PF).

F-Ca/PC/Acid hæmatin.

Pmg_28 S.S. of *Helostomatis deroe* showing the presence of phospholipids in the tegument (T) and parenchymal fibres (PF).

F-Ca/PC/Acid hæmatin.
Pmg-29 T.S. through cyst of *Fibricola indicus*
showing the presence of proteins in the
middle layer of outer cyst wall and outer
and inner layer of inner cyst wall.

Zenker/Mercury bromophenol blue.

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Pmg-30 T.S. through cyst of *Fibricola indicus*
showing the presence of SH group in the
outer and inner cyst wall.

Zenker/Ferric ferricyanide
Pmg-31 T.S. through cyst of *Fibricola indicus* showing the absence of NH$_2$ group of proteins in the inner and outer cyst wall.

Zenker/Ninhydrin Schiff.

Pmg-32 T.S. through cyst of *Fibricola indicus* showing the presence of polysaccharides in the outer and inner cyst walls.

Bouin's/PAS
Pmg-33  T.S. through cyst of *Fibricola indicus* showing the presence of β-metacromasia in the outer and inner cyst wall.

Zenker/Toludine blue

Pmg-34  T.S. through cyst of *Fibricola indicus* showing the absence of glycogen in the outer and inner cyst wall.

Bouin's/Best's carmine
Pmg-35 T.S. through cyst of *Fibricola indicus* showing the presence of mucopolysaccharides in the outer and inner cyst wall.

Zenker/Alcian blue

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Pmg-36 T.S. through cyst of *Fibricola indicus* showing the absence of RNA and DNA from the outer and inner cyst wall.

Zenker/Methyl green pyronin
Pmg. 37 T.S. through cyst of *Fibricola indicus* showing the presence of lipids in the outer and inner cyst wall.

*F*-Ca/PC/Sudan Black B

Pmg. 38 T.S. through cyst of *Fibricola indicus* showing the presence of phospholipids in the outer and inner cyst wall.

*F*-Ca/PC/Acid haematin
Pmg-39 T.S. through cyst of *Fibricola indicus* showing the acidic nature of lipids in the outer and inner cyst wall.

F-Ca/PC/Nile blue
Pmg-40 Cross section of Liver parenchyma showing the normal structure.

Bouin's/Delafield's haematoxylin

Pmg-41 Cross section of Liver parenchyma showing the presence of cyst containing the metacercaria of *Fibricola indicus*.

Bouin's/Delafield's haematoxylin
Pmg-42 Cross section of Liver parenchyma showing the Necrotic tissue.

Bouin's/Delafield's haematoxyler

Pmg-43 Cross section of Liver parenchyma showing the presence of cysts of *Fibricola indicus*. Necrotizing lesions are also seen.

Bouin's/Iron haematoxylin
Cross section of liver parenchyma showing the presence of cysts containing metacercariae at various stages of development.

Bouin's/Delafield's haematoxylin.