HISTOCHEMISTRY

INTRODUCTION :-

Cestodes are made up from the usual tissue contents, proteins, carbohydrates and lipids. But the proportion show a somewhat different patterns from that of most other invertebrates in that the carbohydrates contents tends to unusually high and the protein per unit of body weight relatively low (Archer and Hopkins, 1958, a, b).

The presence of proteins, carbohydrates and lipids though has its existence in cestodes but it has not been studied extensively. The quality and quantity in the whole worms has been observed that we still do not have an idea as to which part they are localized, as the studies are not valid.

Histochemical studies have shown that in cestodes glycogen occurs in expected tissuesites, namely the parenchyma and muscles (Specially those of the suckers), the nervous excretory and reproductive systems are generally free from glycogen (Cheng and Dyckman, 1964 : Hedrick and Daughtery 1957 : Kitejian, Schianazi and Schwabe, 1961 : Read and Simmons, 1963 and Yamo, 1952, c).

Cestodes have substantial carbohydrates requirements and for their normal development and reproduction, carbohydrates must be present in the host diet (Smyth, 1969). The presence of glycogen in C. laticeps was first studied by other Schonbach (1913). Ginetsins Kaya and Uspenskaya (1965) found that the glycogen was primary concentrated in the medullary parenchyma of C. laticeps (from A Brama) with the highest concentration in the posterior part of the body, surrounding the sex glands, which lacked glycogen. The mechanism of uptake of carbohydrates is not known, but the evidence that this take place by active transport as in vertebrates intestinal cells is now impressive (Brand T. Von and Gibbs, 1966 : Phifer, 1960 : and Read 1961).

Lack of glycogen in the diet has profound effect on the development of the parasites. There are for example important differences between the
developmental history of *H. diminutes* and that of *H. nana*, *H. diminuta* grows
an at a more or less constant rate through out its adult life, i.e. it shows no
evidence of sense. In contrast growth of *H. nana* is rapid for only 10-12 days
after reaching the intestine and thereafter new segments are produced more
slowly. Carbohydrates requirements therefore, would be high only during the
early phases of establishment and strobilar differentiation, *H. Citelli* appears
to be intermediate between *H. nana* and *H. diminuta* both in carbohydrate
deficiency (Read, Schiller and Phifer, 1958). Similar work on Raillietina in
birds, Read (1942) *oochoristica* in rodents and *lecestroarynchus* in dog fish.
Read (1957) confirms that carbohydrates in the host diet is a necessity for
cestode growth.

The quality of host carbohydrates also has an effect on growth, as does
the level of infection in a host i.e. there is a crowding effect related to the
number of worms present. In host rats fed on starch, limited starch or sucrose,
the size of the worm decreased in proportion as the worm burden increased in
all three cases (Read and Phifer, 1959).

Since proteins forms a substantial part of the normal diet of a
vertebrate, the intestine provides for cestode an environment rich in proteins
and related breakdown products, polypeptides, dipeptides and amino acids
(Smyth, 1969).

Fractionation of tissue proteins of cestodes has been carried out in only
a few species, *Echinococcus granulosus* (G melik, 1955; Pozzi and Pirosky,
c.), *T. Saginata* (Machnika - Roguska, 1961). Some of these proteins as usual
in that they have been found to be conjugated with other tissue substances
such as glycogen cerebrocides or bile acids.

The structural proteins reported in cestodes are keratin, which makes
up the hooks and embryophores in taeniid cestodes and selerotin which forms
the egg capsule in pseudophyllida and possibly other groups (Smyth, 1969).

The amino acids occurring in the tissue of a number of adult and larval
cestodes have been examined and data are available for *H. diminuta* (Aldrich,
The lipid metabolism of cestodes has been examined to a limited extent, and most studies have been confined to quantitative and qualitative examination of lipid content and its distribution in the tissues (Smyth, 1969).

The role of lipids in the cestode metabolism is not clear and there is no evidence that the lipids act as energy reserves in cestodes as they do in nematodes. In *H. diminuta*, lipids tend to be more abundant in the most posterior proglottids (Fairlairn Wertheim, Harper and Schiller, 1961). The higher content of lipid in mature proglottids has led to the view that much of this lipid largely represents waste products of metabolism (Brand T. Von. 1952 and 1966).

Cholesterol has been identified in *T. Saginata* (C. Melik and Briski, 1953), *H. diminuta* (Fairbairn, Wertheim, Harpller and Schiller, 1961), *Hydrotigera taeniaeformis*, *M. expansa* (Thomson, Mosetting and Brand T. Von, 1960), D. Latum (Read and Simons, 1963) and *E. granulosus* (Gmelik, 1953 b.), Arme (1966) studied some enzymes of Ligula intestinalis is Bogtish (1963), studied *M. microstoma histochemically*, comparative account of lipids in the legument was observed by Bogoyanlensleii Ya.

kyu K. and Nikitine (1979), Cheah (1967) studied Moniezia

expansa surface carbohydrates were observed by Prashad and Guraya (1977). Histochemical studies on *Railletina* . (R.) Johri was made by Roy (1980).

Histochemistry makes the internal and functional aspects of the structure more valid.
The author has used different fixative and stains for the demonstration of glycogen protein and lipids.

The fixative used for glycogen is Carnoy’s fluid and the stain is Best’s carmine (Best, 1906) preparation of carnoy’s fluid.

- **Ethyl alcohol** - 60ml.
- **Chloroform** - 30ml.
- **Glacial acetic acid** - 10ml.

### PREPARATION OF SOLUTIONS

- **Carmine Stock Solution** :-

  This was prepared by gently boiling 2gm. of carmine powder, 1gm. Potassium carbonate and 5gm. Potassium chloride in 60ml. distilled water. It was cooled and filtered to the filtrate 20ml. of liquor ammonia was added and stored.

- **Carmine staining solution** :-

  This staining solution was prepared by diluting 15ml. of stock solution with 12.5 ml. of liquid ammonia and 12.5 ml. of methyl alcohol. This solution lasts for 2-3 weeks.

- **Best’s differentiator** :-

  - **Absolute alcohol** - 8ml.
  - **Methyl alcohol** - 0.4ml.
  - **Distilled water** - 10ml.

  The fixative used for protein determination to Carnoy’s fluid and the stain to mercury Bromophenol blue (Alter Bonhag, 1955).

  - 1% HgCl₂ and 0.50% Bromophenol blue, 2% aqueous acetic acid.

  The fixative used for the lipid determination is formal calcium and the stain is Sudan black B and sections were mounted in glycerine jelly (After Mc Manus, 1946).
• Preparation of fixative formal calcium :-
  40% formaldehyde - 10 ml.
  10% calcium chloride - 10 ml.
  Distilled water - 80 ml.

To maintain the PH (4-8), pieces of chalk are added.

• Preparation of staining solution :-
  Sudan black ‘B’ Solution is prepared in 70% alcohol, dissolved the Sudan black ‘B’, powder in 70% alcohol up to saturated level and used for staining.

• Glycerine Jelly :-
  Distilled water - 40ml.
  Gelatin - 80 gm.
  Heated for 2 hrs.
  Glycerine - 50 ml.

  Phenol-----------------------1ml Kept in hot water bath for 10-15 minutes.

GLYCOGEN CONTENT IN RAILLETINA

Experimental study of the histochemistry of Railletina tetragona Molin was carried out in the laboratory at the Sangamner in the month of October 2008. Twelve cestode parasites were collected from the intestine of Gallus domesticus at Loni, Tal. Rahata, Dist.Ahmednagar, M.S., India.

The worms were washed with tap water and few of them were fixed in Carnoy’s fluid for determination of glycogen and protein content of the detection of Lipid content.

After worms were removed from the fixative, washed with water, dehydrated through alcoholic grade and embedded in paraffin wax (58- 60°C) and blocks were prepared. The blocks were cut at 7mm thickness with the help of microtome machine. The sections were taken on the slide. The
sections were deparaffinised and passed through alcoholic grades (absolute alcohol to water) for hydration, then stained with Ehrlich’s Haematoxylin for 5 minutes rinsed rapidly in 3-5% acid alcohol and water. They were stained with Best’s carmine for half an hour and differentiated in Best’s differentiator for a minute.

Then slides were washed with 70% alcohol and passed (dehydrated) through 90%, 100% alcohol, cleared in xylene and mounted in D.P.X. Glycogen stained pink red to red in colour.

Prepared selected slides of *Railletina tetragona Molin* when observed under microscope showed that the worm contains high concentration of the glycogen in its body.

The immature segments show high concentration of the glycogen integument up to muscle layer. There is high traces of glycogen, particularly in the center of the segment, peripheral region and ovary, but in testes moderate quantity of glycogen is seen. The glycogen concentration is also seen in the lateral parenchyma.

The mature segment shows relatively moderate amount of glycogen in the longitudinal muscles and peripheral region and relatively more concentration in the reproductive organs.

Thus it can be concluded that *Railletina* has glycogen deposited in quantity throughout the body for metabolic activity.

**GLYCOGEN CONTENT IN SENGA**

Experimental study of the histochemistry of *Senga sakurensis* was carried out in the laboratory at the Sangamner in the month of October 2008. Twelve cestode parasites were collected from the intestine of *M.armuts* M.S., India.
The worms were washed with tap water and few of them were fixed in Carnoy’s fluid for determination of glycogen and protein content of the detection of Lipid content.

After worms were removed from the fixative, washed with water, dehydrated through alcoholic grade and embedded in paraffin wax (58- 60°C) and blocks were prepared. The blocks were cut at 7mm thickness with the help of microtome machine. The sections were taken on the slide. The sections were deparaffinised and passed through alcoholic grades (absolute alcohol to water) for hydration, then stained with Ehrlich’s Haematoxylin for 5 minutes rinsed rapidly in 3-5% acid alcohol and water. They were stained with Best’s carmine for half an hour and differentiated in Best’s differentiator for a minute.

Then slides were washed with 70% alcohol and passed (dehydrated) through 90%, 100% alcohol, cleared in xylene and mounted in D.P.X. Glycogen stained pink red to red in colour.

Prepared selected slides of *Senga sakurensis* when observed under microscope showed that the worm contains high concentration of the glycogen in its body.

The immature segments show high concentration of the glycogen integument upto muscle layer. There is high traces of glycogen, particularly in the center of the segment, peripheral region and ovary, but in testes moderate quantity of glycogen is seen.. The glycogen concentration is also seen in the lateral parenchyma.

The mature segment shows relatively moderate amount of glycogen in the longitudinal muscles and peripheral region and relatively more concentration in the reproductive organs.

Thus it can be concluded that *Senga sakurensis* has glycogen deposited in quantity throughout the body for metabolic activity.
Glycogen content in *Raillietina (Raillietina) tetragona* Molin, 1858

Immature proglottid  Mature proglottid

Glycogen content in *Senga sakurensis* sp. nov

Immature proglottid  Mature proglottid
PROTEIN CONTENT IN RAILLIETINA

Out of the 12 worms collected from Gallus domesticus at Sangamner, Dist. Ahmednagar, M.S., India. Some worms were already been fixed in Carnoy’s fluid, fixative as mentioned in the earlier chapter of glycogen estimation of the same worm. Few of them were utilized for the protein determination.

The worms fixed in the fixative was removed and washed with tap water and dehydrated through alcoholic grades, embedded in paraffin wax (58-60°C) M.P. and blocks were prepared. Sections were taken at 7mm with the help of microtome machine, sections were taken on slides and then deparaffinised with xylene, hydrated and stained with mercury bromophenol blue for about 1½ to 2 Hrs. transferred in 1% aqueous acetic acid for 5 min. washed directly with the tertiary butyl alcohol, cleared in xylene and mounted in D.P.X. to prepare permanent studies were selected for histochemical studies.

Observation of slides showed that proteins are darkly stained in immature and mature segments of the worms. In immature protein is in high concentration at the lateral circular parenchyma and in muscle layer.

In mature segment the amount of protein is high in ovary and testes i.e. medullary region. This integument contains relatively less amount of protein.

Thus from above observation, it is clear that in Railletina tetragona Molin have relatively high content of protein which is utilized for their metabolic activities.
PROTEIN CONTENT IN SENGA

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Thus from above observation, it is clear that in *Senga sakurensis* have relatively high content of protein which is utilized for their metabolic activities.
Protein content in *Raillietina (Raillietina) tetragna* Molin, 1858

- Immature proglottid
- Mature proglottid

Protein content in *Senga sakurensis* sp. nov

- Immature proglottid
- Mature proglottid
LIPID CONTENT IN RAILLETINA

The cestode parasites of Railletina tetragona Molin were collected from the intestine of Gaullus domesticus and were fixed in formal calcium fixative, for determination of Lipid content as already described in glycogen content chapter.

After worms were removed from the fixative washed with water and dehydrated through alcoholic grades. Then embedded in paraffin wax (58-60°C M.P.) to prepare blocks. The blocks were cut on microtome at 7mm thickness. Sections were taken on slides. These sections were deparaffinised in xylene and brought to 70% alcohol through alcoholic grades, stained in Sudan black ‘B’ saturated solution prepared in 70% alcohol for about 30-60 min. and were dehydrated in alcoholic grades cleared in xylene and mounted in glycerine jelly. Lipid indicates their presence by black-brown colour.

Observations of the selected slides reveals that average amount of Lipid is present, which stained brownish black in colour with stain Sudan black ‘B’.

In the mature segment moderate quantity of Lipid present in peripheral region i.e. in muscle layer. It is relatively high in the tegument in tested and ovary as well as in medullary part of segment.

Thus it can be concluded that Railletina tetragona Molin has stored average amount of Lipid Content in its tissue for various metabolic activities.

LIPID CONTENT IN SENGA

The cestode parasites of Sanga sakurensis were collected from the intestine of M. armatus and were fixed in formal calcium fixative, for determination of Lipid content as already described in glycogen content chapter.

After worms were removed from the fixative washed with water and dehydrated through alcoholic grades. Then embedded in paraffin wax (58-
60°C M.P.) to prepare blocks. The blocks were cut on microtome at 7mm thickness. Sections were taken on slides. These sections were deparaffinised in xylene and brought to 70% alcohol through alcoholic grades, stained in Sudan black ‘B’ saturated solution prepared in 70% alcohol for about 30-60 min. and were dehydrated in alcoholic grades cleared in xylene and mounted in glycerine jelly. Lipid indicates their presence by black-brown colour.

Observations of the selected slides reveals that average amount of Lipid is present, which stained brownish black in colour with stain Sudan black ‘B’.

In the mature segment moderate quantity of Lipid present in peripheral region i.e. in muscle layer. It is relatively high in the tegument in tested and ovary as well as in medullary part of segment.

Thus it can be concluded that *Sanga sakurensis* has stored average amount of Lipid Content in its tissue for various metabolic activities.
PLATE - 10

Lipid content in *Raillietina (Raillietina) tetragona* Molin, 1858

[Images of immature and mature proglottids]

Lipid content in *Senga sakurensis* sp. nov

[Images of immature and mature proglottids]
• **Results :-**
  
  Glycogen stains pink red to red in colour.

• **Results :-**
  
  Protein stains deep clear blue colour.

• **Results :-**
  
  Lipid stains black or blue if present in sufficient quantity. Even a brownish black stain may be indication of the presence lipid or lipoprotein.

  With the help of these three staining techniques, carbohydrates, proteins and lipids concentrated in the tissue were visible.