Chapter- III

Biochemistry

Introduction

Material and methods

Result and discussion

Conclusion

Summary of chapter-III
BIOCHEMISTRY
GLYCOGEN ESTIMATION IN CESTODES

INTRODUCTION:

The cestode parasites utilize the food from the intestinal tract of the host, so their metabolism depends on feeding habits and the rich nourishment available in the gut of the host. Parasites take nourishment for their normal development and growth from host. The metabolic and in vitro studies suggest that a complex nutritional relationship occurs in the cestodes and they are capable of fixing CO_{2} Thus it is clear that the parasites use the waste metabolic materials from the host intestinal mucosa very efficiently. Where as there are other species, which reveal to be capable of taking in the nutritional material by direct contact with the mucosal wall.

The main carbohydrates reserve in parasitic helminths is “Glycogen” which is a typical energy reserve of helminths inhabiting biotopes with low oxygen tension. The main polysaccharide in cestodes is glycogen, closely resembling mammalian glycogen. The early work of Bernard Claude (1859) and Foster (1856) demonstrated the occurrence of glycogen in helminths.

It has been known for more than hundred years, (Barnard, 1889; Foster, 1865) that parasitic worms contains polysaccharides, weinland’s classic work (Weinland, 1901 a, b) illustrated that the metabolism of intestinal worms is characterized by the fermentation of carbohydrates. Following the work of these and other pioneers who studies some phase of the carbohydrate relationship of the parasites (Von Brand, 1970), it has become obvious that many endoparasites have a pronounced carbohydrate metabolism.

Sufficient literature is present for parasitic worms in relation to the distribution of carbohydrates. The quantitative values found in previous and many of the recent literature e.g. Weinland (1901 b), Schulte (1917), Von Brand, (1934), Salisbury and Anderson (1939), Daugherty and Taylor (1956), Goodchild and Vilar Alvarez (1962) and others have been obtained by rather unspecific chemical method, these often give higher values than those obtained by mean of an enzymatic procedures, may explain for example, the widely differing glucose values reported by Fairbairn (1958) and Lopez-
Gorge and Monteolive (1964) for *Moniezia expansa*. Reliable quantitative or semi quantitative data has also been obtained by means of paper chromatography.

The glucose content of various helminths fluctuates considerably and there is no link of peculiarities of the habitat. This reveals the glucose concentration in the tissue of *Taenia taeniaeformis*, which rises by as much 100-200mg/100mg. On incubating in vitro in glucose containing medium (Von Brand et al 1964), but it also rises in worms incubated in sodium free salines, which do not permit glucose absorption. In this instance enlarged tissue glucose has been presumably derived from a glycogen breakdown (von Brand and Gibbs, 1966). It is possible that glucose is not evenly distributed along the strobila of *H. diminuta* (Goodchild, 1961), but whether nutritional factors play a role in it is not known.

Glucose is said to be an important energy source for helminths inhabiting the alimentary tract of vertebrates. It is generally believed that helminths absorb glucose against a concentration gradient and use their endogenous carbohydrates only as an energy source when it is unobtainable from its media. Daugherty et al., (1956), Fairbairn, Werthein, Harpur and Schiller (1961), Markov (1939), Read, Rothman (1957 b) have pointed out that the cestodes have very high rate of carbohydrates, high rate of transport of exogenous glucose into the body, high rate of utilization of endogenous carbohydrate and high rate of glycogenesis.

The cestodes parasites as a group store relatively large quantities of polysaccharides, which in most cases have assumed to be glycogen, Read (1949 b), Reid (1942).

The literature at our disposal discloses that the carbohydrate plays an important role in cestodes, than in most other parasites worms, which are distinguished by different growth patterns. These carbohydrates are utilized exogenously, their mechanism of the uptake is not known but the evidence indicates that the active mechanism undoubtedly is entangled in the carbohydrate transport of helminths. The cestodes *H. diminuta* (Phifer, 1960 a, b, c; Fisher, 1965), *T. taeniaeformis* (Von Brand et al., 1964) and *Calicobothrium verticillatum* (Fisher, 1965) as well as the Acanthocephalan *Polymorphus minutus* (Crompton and Lackwood, 1968) absorb glucose against concentration gradients. Further more typical inhibitors of active transport e.g. phloridizin interferes effective with the
glucose uptake of cestodes. (Phifer 1960 a, Lauris, 1961, Von Brand et al., 1964) and at least in *T. taeniaeformis*, glucose absorption has an absolute sodium requirement (Von Brand et al., 1964, Von Brand and Gibbs 1966), apparently corresponding closely to the sodium pump of vertebrate tissue.

The glycogen content of cestodes fluctuates over a wide range due to factors such as season, physiological state of the host, the time of autopsy, strain of the host, rate of infection and to some extent on the stage of the life cycle. In few cestodes developmental history changes, the growth of parasites is rapid at the first 18-24 hours and then slows down, even if the concentration is very high as it was in the early phase. It has been observed that the same in *H. diminuta* increase from 15% of the dry substance in 5 and 7 day old worms to 37% in 13 and 16 days old specimens (Mettrick and Cannon, 1970), it has been observed that the uptake of glucose is very much effective when CO2 is present in the surrounding than when it is absent.

A number of workers have reported the content of glycogen in different helminth parasites. The terms of percentages the glycogens content of a few parasites are given in terms of percentage. In *Taenia taeniaeformis* 2.5 to 5.6% (Von Brand et al., 1968), in *Hymenolepis diminuta* from 1.1 to 9.3% (Fairbairn et al., 1961) 16% in *Schistocephalus Solidus* larva (Hopkins, 1950) respectively.

The species which have been previously experimented by different workers for the carbohydrate metabolism are *Taenia crosiceps*, *T. pisiformes*, *T. saginata*, *Moniezia expansa*, *M. bidenti*, *Echinococcus granulossus*, *Diphylidium canium*, *Bothriocephalus gowkongensis*, *phyllobothrium foliatum*, *H. diminuta*, *H. utelli*, *H. nana* and the genus *Oochoristica*, *Raillietina* etc.

**ESTIMATION OF GLYCOGEN**

**Principle:**

The Anthrone reaction is rapid method for the determination of hexoses, aldopentoses and hexuronic acids, either free or present in polysaccharides. The blue green solution shows absorption maximum at 620µm.
Reagents:

1) 30% KOH: Dissolve 30gms of KOH (Potassium Hydroxide) in 100 ml of distilled water.
2) 2% Na₂SO₄ solution: Dissolve 2gms of sodium Sulphate (Na₂SO₄) in 100ml of distilled water.
3) 100% ethyl alcohol.
4) Anthrone reagent: Dissolve 150mg of Anthrone in 100ml of Conc.H₂SO₄ (88%)
5) Standard stock solution: 100mg of glucose in 200ml of distilled water.
6) Blank solution: 1ml distilled water+ 5ml Anthrone reagent.
GLYCOGEN ESTIMATION IN
LYTOCESTUS VYASAEI N.SP.

MATERIAL AND METHODS:

The twelve fresh water fish *Clarias batrachus* (Linnaeus) were brought to the laboratory, killed by pithing brain and dissected out intestine, carefully intestine cut open for the collection of cestode parasites. Out of twelve intestines, seven intestines were infected with cestode parasites. Identical worms were separated and kept separately, by observing under the microscope and few of them were fixed in 4% formalin for taxonomic studies. Taxonomical observations turned out them, to be the species of the genus *Lytocestus vyasaei* n.sp. The small pieces of infected and non infected intestine were also collected for estimation of glycogen and is estimated by using DeZwaan and Zandee (1972) method.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60 °C for twenty-four hours, for drying and then taken the dry weight of material and prepared a powder with the help of mortar pestle. This powder weighed 100.00mgs on a sensitive balance and homogenized in a mortar and pestle by adding 1ml of 30% KOH and taken in centrifuge tube kept in boiling water bath for 3 to 5 minutes, cooled it at room temperature, then added 0.2 ml of 2% Na₂SO₄ solution. Add to the same 6 ml of absolute alcohol by stirring with a glass rod and keep it in refrigerator overnight for formation of carbohydrate ppt. This carbohydrate ppt solution centrifuged for 15 minutes at 3000 RPM and discards the supernatant (glycogen settled at bottom) and dissolve residue by adding 10 ml of distilled water, in each 5 ml of Antherone reagent was added and mixed, then kept in it boiling water bath for 10 minutes and immediately cooled and reading were taken with the help of Colorimeter at 620μm filter.

Similarly for standard glucose concentration was used (100mg of glucose in 200ml of distilled water) and read the optical densities.
RESULT AND DISCUSSION:

The glycogen content in *Lytocestus vyasaei* n.sp. and infected and uninfected intestinal tissues in the host *Clarias batrachus* was calculated by statistical analysis.

**Table –1.** glycogen content (mg/100mg of dry tissue) of *Lytocestus vyasaei* n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Glycogen content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clarias batrachus</em> L.</td>
<td><em>Lytocestus vyasaei</em> n.sp.</td>
<td>35.81±3.19</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>16.79±1.06</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>23.54±2.13</td>
</tr>
</tbody>
</table>

The values of glycogen content for whole worms, *Lytocestus vyasaei* n.sp. with their respective hosts intestinal tissues are given in table no. 1 (Histogram 1). The glycogen content of *Lytocestus vyasaei* n.sp. was higher as compared with the infected and uninfected intestinal tissues as 35.81±3.19, 16.79±1.06 and 23.54±2.13 mg/100mg dry tissue respectively.

*Lytocestus vyasaei* n.sp. is an intestinal parasites and environmental oxygen is not available. These parasites depend on anaerobic carbohydrate metabolism to obtain the energy required. As there was no possibility of aerobic source of energy, a regular supply of glycogen was necessary hence; large quantities of polysaccharides are stored which can be oxidized to yield (ATP) Adenosine triphosphate.

The values of glycogen content of *Lytocestus vyasaei* are comparable with those of *Hymenolepis diminuta* (Fairbairn et al., 1961) and *Taenia taeniformis* (Von T. Brand et al., 1968). The glycogen content was higher than their respective host tissue. Similar trends of glycogen had been reported by Malhotra (1992), the decrease in glycogen content of intestinal tissues of infected rats and their uninfected tissues.
GLYCOGEN ESTIMATION IN
GANGESIA (GANGESIA) RAMKAEI N.SP.

MATERIAL AND METHODS:

The ten fresh water fish Wallago attu (Bleeker) were brought to the laboratory, killed by pithing brain and dissected out intestine, carefully intestine cut open for the collection of cestode parasites. Out of ten intestines, four intestines were infected with cestode parasites. Identical worms are separated and kept separately by observing under the microscope and few of them were fixed in 4% formalin for taxonomic studies. Taxonomical observations turned out them, to be the species of the genus Gangesia (Gangesia) ramkaei n.sp. the small pieces of infected and non infected intestine were also collected for estimation of glycogen and is estimated by using DeZwaan and Zandee (1972) method.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then taken the dry weight of material, prepared a powder with the help of mortar pestle. The powder weighed 100.00mgs on a sensitive balance and homogenized in a mortar and pestle by adding 1ml of 30% KOH and taken in centrifuge tube and kept in boiling water bath for 3 to 5 minutes, cooled it at room temperature, then added 0.2 ml of 2% Na₂SO₄ solution. Add to the same 6 ml of absolute alcohol by stirring with a glass rod and keep it in refrigerator overnight for formation of carbohydrate ppt. This carbohydrate ppt solution centrifuged for 15 minutes at 3000 RPM and discards the supernatant (glycogen settled at bottom) and dissolve residue by adding 10 ml of distilled water, in each 5 ml of Antherone reagent was added and mixed, then kept in it boiling water bath for 10 minutes and immediately cooled and reading were taken with the help of Colorimeter at 620μm filter.

Similarly for standard glucose concentration was used (100mg of glucose in 200ml of distilled water) and read the optical densities.
RESULT AND DISCUSSION:

The glycogen content in *Gangesia (Gangesia) ramkai* n.sp. and infected and uninfected intestinal tissues in the host *Wallago attu* was calculated by statistical analysis.

**Table - I.** Glycogen content (mg/100mg of dry tissue) of *Gangesia (G.) ramkai* n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Glycogen content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wallago attu</em> (Blekker)</td>
<td><em>Gangesia (G.) ramkai</em> n.sp.</td>
<td>31.52±1.06</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>22.56±0.37</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>27.59±0.21</td>
</tr>
</tbody>
</table>

The values of glycogen content for whole worms, *Gangesia (G.) ramkai* n.sp. with their respective hosts intestinal tissues are given in table no. 2 (Histogram 2). The glycogen content of *Gangesia (G.) ramkai* n.sp. was higher as compared the infected and uninfected intestinal tissues like 31.52±1.06, 22.56±0.37 and 22.59±0.21 mg/100mg dry tissue respectively.

Several earlier workers have provided evidence from their host deficiency and starvation of experiments that the carbohydrates are of vital importance in the metabolism of cestodes (Read and Rothman, 1957b). Mead and Roberts (1972) recorded a net loss to the carbohydrate diet of rats due to *H. diminuta* infection. It was emphasized by Read (1970) that the presence *H. diminuta* in the rat intestine modified the physiology of carbohydrate digestion and as a result depletion in the rate of absorption of nutrients was recorded (Podesta and Mettrick, 1977). Smyth (1976) did not rule out the possibility of identical structural properties of various glycogens found in cestodes and their vertebrates hosts. Von Brand (1973) suggested that the tapeworms obtain their nutrient supply either from intestinal mucosa or exocrine-enteric circulation of host. Hence it is considered view of the author that the observed fall result in glycogen content in the infected host tissues might have occurred due to physiological dysfunction occurring in the intestinal tissues of host which thereby results into decreased rate of supply of glucose to the intestine.
Figure no.11. Glycogen content in *L. vysaei* n.sp., infected and uninfected intestinal tissues of *Clarias batrachus* (mg/100mg dry tissue).

Figure no.12. Glycogen content in *G. (G) ramkaei* n.sp., infected and uninfected intestinal tissues of *Wallago attu* (mg/100mg dry tissue).
PROTEIN ESTIMATION IN CESTODES

INTRODUCTION:

Proteins have many different biological functions; they are ubiquitous in their distribution and there is really no satisfactory scheme of classifying them. The largest groups of proteins are the enzymes, of which nearly 2000 different ones have been described. Proteins are also involved in contractile systems, in transport, as protective agents, toxins, hormones, and amino acid reserves and as important structural components. In parasitic helminths, protein usually constitutes between 20 and 40% of the dry weight, but values as high as 70% of the dry weight.

Proteins are absorbed by the parasites by diffusion and transmission. It is naturally available from the host tissue as there is no media to acquire proteins in parasites; these proteins are naturally available from the host tissue. These worms utilize different degree of protein for producing energy. Literature reveals that the parasites able to adopt themselves to the parasitic mode of life due to protein metabolism. These parasites excrete amino acids as their end product. For these worms protein are very essential, therefore parasites do not depend on the host’s dietary proteins. If host’s proteins are removed from the diet, even the amino acids occur in the intestinal lumen. When rats were given protein free diet then also *Hymenolepis diminuta* (Phifer, 1960 a, b, c.; Fisher, 1965) developed fantastically. Mettrick and Munro, (1965) where as low protein diet given to rats, while favoring the migratory phase of *Nipponstroglypus basiliensis* proved some what unfavorable to the adult worm (Clarke, 1968) but indirect rather than direct influence was probably involved Clarke, (1968)

The essential and non-essential amino acids are required, proteins are also digested at the host parasites interface by the activity of proteolytic enzyme and the cestode tegument secretes these amino acids are absorbed by active transport but some amino acids try to inhibit the uptake of others. Where as some have no effect. In *Hymenolepis diminuta* there was interference by amino acids if the diet contained an incomplete protein or there was imbalance in dietary amino acids (e.g. Casein or Zein). It may be possible (Mettrick 1971) that inhibition might be due to the tendency of cestode to acquire carbohydrates (Polyfunctional carrier system) or changes in the molar ratios of
intestinal amino acids. Though other scientists (Good Child and Dennis 1965, Hopkins and Young, 1967) did not get the same results but it was clear to all that due to the parasitic life led by *Hymenolepis diminuta* the composition of the amino acid pool and other parameters is changed (Mettrick, 1971 b) this can have an influence on the response of the parasites to an altered host diet. Further studies reveal that the molar ratios of amino acids differ with differing dietary proteins, as well as in various patterns of the gut (Mettrick, 1970) their molar ratios are factors in determining host.

Many workers have gone through the studies of protein metabolism in various cestode parasites. They are amino acid oxidises in *Hymenolepis diminuta* and some of changes in host, physiology by Daugherty (1955). Studies on protein complexes of the cestode *Raillietina cesticillus* by Kent (1957 b), amino acids in the hydatid fluid plasma layer of *Echinococcus* by Karvavica et al.; (1959 b), urea formation and urea cycle present in the cestode, *Hymenolepis diminuta* by Campbell (1963 a) absorption and digestion of amino acid in the tapeworm *Anoplocephala magna* by Karvavica et al. (1959a) and the estimation in *Phyllobothrium foliatum* has been worked out. Sidorov (1980) made comparative investigation of proteins composition and *Eubothrium crassum* and the host. Ganzalez (1978) worked on serum protein in animals, which were infected, with parasitic helminthes.

**PROTEIN ESTIMATION BY LOWRY’S METHOD**

**Principle:**

The first step involves formation of a copper protein complex in alkaline solution. This complex then reduces a phosphomolybdic-phosphotungastate reagent to yield an intense blue colour. This assay procedure is much more sensitive than the biuret method but is more time consuming. The only precaution to be observed when performing this assay concerns addition of the Folin’s reagent. This reagent is stable only at acidic pH; however, the reduction indicated above occurs only at pH 10. Therefore, when Folin’s reagent is added to the alkaline copper protein solution, mixing must occur immediately so that the reduction can occur before the phosphomolybdic-phosphotungastate (Folin’s) reagent breaks down.
Reagents:

1) Lowry’s ‘A’ solution: Dissolve 2gms of Na₂CO₃ in 100ml of NaOH solution
   (400mg of NaOH in 100ml of distilled water = 0.1 N NaOH.)

2) Lowry’s ‘B’ solution: B₁ = 1% Copper Sulphate (CuSO₄)
   B₂ = 2% sodium Citrate

3) Lowry’s ‘C’ solution: 1ml of B₁ + 2ml of B₂ diluted to 100ml with Lowry’s ‘A’
   solution (Always prepare fresh)

4) 10% TCA solution: Dissolve 10gm of Trichloroacetic acid (TCA) in 100ml of
   distilled water.

5) 1 N NaOH: Dissolve 4gm of NaOH (sodium Hydroxide) in 100ml of distilled
   water.

6) Folin Phenol reagent: This reagent is diluted by distilled water in preparation of
   1:1.

7) Standard stock solution: 50mg of BSA (Bovine Serum Albumin) in 100ml of 1 N
   distilled water.

8) Blank solution: 0.1ml (1 N NaOH) + 4ml of Lowry’s ‘C’ solution + 0.4ml of Folin
   Phenol.
PROTEIN ESTIMATION IN
LYTOCESTUS VYASAEI N.SP.

MATERIAL AND METHODS:

The estimation of protein content in the cestode parasites was carried out by Lowry’s method.

Eight *Clarias batrachus* fish were brought to the laboratory killed by pithing brain and dissected. The intestine carefully dissected and observed for collection of cestode parasites, five of them were found to be heavily infected with cestode parasites, with small pieces of infected and non-infected intestine was also collected to find out the protein content of these tissues.

The identical worms were separated and kept separately by observing them under microscope. Few of them were kept in 4% formalin for the identification. These worms later stained with Grenachers Borax Carmine and identified as *Lytocestus vyasaei* n.sp.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue was recorded. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then taken the dry weight of material, prepared a powder with the help of mortar pestle. The powder weighed 100.00mgs on a sensitive balance and then it is homogenized in a mortar and pestle with 1 ml of 10% TCA solution and transferred in centrifuge tube. Then it is centrifuged for 15 minutes at 3000 RPM. Supernatant is discarded and residue is taken and added 10 ml of 1 N NaOH for dissolved the ppt. Then taken 0.1 ml above solution and add 4 ml Lowry’s ‘c’ solution, then it add 0.4 ml of Folin Phenol reagent and this test tubes was kept for half an hour at dark place, until the blue colour developed. Then this colour was read on the colorimeter with 530μm filter to note the optical density to calculate the protein content.

Bovine Serum albumin was used for standard (10mg of BSA in 10ml of 1 N NaOH) and read the optical densities.
RESULT AND DISCUSSION:

The protein content in *Lytocestus vyasaei* n.sp. and infected and uninfected intestinal tissues in the host *Clarias batrachus* was calculated by statistical analysis.

**Table -1.** Protein content (mg/100mg of dry tissue) of *Lytocestus vyasaei* n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clarias batrachus</em> L.</td>
<td><em>Lytocestus vyasaei</em> n.sp.</td>
<td>27.05±0.75</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>10.16±0.75</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>19.25±1.49</td>
</tr>
</tbody>
</table>

The results are represented by histogram no.3 the protein content of *Lytocestus vyasaei* n.sp. 27.05±0.75 was higher than the infected and uninfected intestinal tissues. The uninfected tissue 19.25±1.49 was higher than the infected tissue i.e. 10.16±0.75 mg/100mg.

Proteins occurs in parasites the presence and utilization of proteins are reported in cestodes by various authors, Campbell (1960), Goodchild, (1961), Goodchild and vilar-Alvarez (1962), Reid, (1942), Hopkins, (1950), and Von Brand and Bowman (1961).

Goodchild (1961) reported protein content in *Hymnolepis diminuta* isolated from adequately fed rats contained 32% protein, while the corresponding figure for worms taken from starved rats was 59.5%.

In the present study protein content in *Lytocestus vyasaei* n.sp. is observed it is 27.05%, but their host tissues, infected and uninfected intestinal tissue protein content is less compared to the parasite tissue.
PROTEIN ESTIMATION IN

GANGESIA (GANGESIA) RAMKAEI N.SP.

MATERIAL AND METHODS:

The estimation of protein content in the cestode parasites was carried out by Lowry's method.

Five *Wallago attu* fish were brought to the laboratory, killed by pithing brain and dissected. The intestine carefully dissected and observed for collection of cestode parasites, three of them were found to be heavily infected with cestode parasites, with small pieces of infected and non-infected intestine was also collected to find out the protein content of these tissues.

The identical worms were separated and kept separately by observing them under microscope. Few of them were kept in 4% formalin for the identification. These worms later stained with Grenachers Borax Carmine and identified as *Gangesia(Gangesia) ramkaei* n.sp.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue was recorded. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then taken the dry weight of material and prepared a powder with the help of mortar pestle. The powder weighed 100.00mgs on a sensitive balance and then it is homogenized in a mortar and pestle with 1 ml of 10% TCA solution and transferred in centrifuge tube. Then it is centrifuged for 15 minutes at 3000 RPM. Supernatant is discarded and residue is taken and added 10 ml of 1 N NaOH for dissolved the ppt. Then taken 0.1 ml above solution and add 4 ml Lowry's 'c' solution, then it add 0.4 ml of Folin Phenol reagent and this test tubes was kept for half an hour at dark place, until the blue colour developed. Then this colour was read on the colorimeter with 530µm filter to note the optical density to calculate the protein content.

Bovine Serum albumin was used for standard (10mg of BSA in 10ml of 1 N NaOH) and read the optical densities.
RESULT AND DISCUSSION

The Protein content in Gangesia (Gangesia) ramkaei n.sp. and infected and uninfected intestinal tissues in the host *Wallago attu* was calculated by statistical analysis.

**Table –I.** Protein content (mg/100mg of dry tissue) of Gangesia (G) ramkaei n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Wallago attu</em> (Bleeker)</td>
<td><em>Gangesia (G.) ramkaei</em> n.sp.</td>
<td>22.72±0.75</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>7.14±0.74</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>17.09±2.25</td>
</tr>
</tbody>
</table>

The values of protein content shows that the whole worms of Gangesia (Gangesia) ramkaei n.sp. was highest (22.72±0.75) as compared infected and uninfected intestinal tissues. The protein content of uninfected intestinal tissue (17.09±2.25) is higher than the infected tissue (7.14±2.25).

The protein content of helminth parasites fluctuates over a wide range due to factors such as seasons, physiological state of the host, the time of autopsy, strain of the host, rate of infection and the stage in the life cycle.

A number of workers have reported the content of protein in different helminth parasites. The term of percentages. The protein content of a few parasites are given in terms of percentage. In *Taenia taeniaeformis*, larval 27-29%, adult 45% (Von Brand and Bowman, 1961); *Moniezia expansa*, 22% (Campbell J.W., 1969) and 36% in *Schistoscephalus solidus* (Hpkins 1950) respectively.

The protein from a sustantial part of the normal diet, the intestine provides for cestode, an environment rich in proteins and related breakdown products, polypeptides, dipeptides, and amino acids (Smyth 1969). In parasitic helminthes, protein usually constituents between 20 and 40% of the dry weight, but values as high as 70% of the dry weight. In the present study protein content of the cestode Gangesia (G.) ramkaei n.sp. is highest (22.72%) as compared to infected and uninfected intestinal tissue.
**Figure no.13.** Protein content in *L. vyasaei* n.sp., infected and uninfected intestinal tissues of *Clarias batrachus* (mg/100mg dry tissue).

**Figure no.14.** Protein content in *G. (G) ramkaei* n.sp., infected and uninfected intestinal tissues of *Wallago attu* (mg/100mg dry tissue).
LIPID ESTIMATION IN CESTODES

INTRODUCTION:

Lipids are heterogeneous group of compounds with similar physical properties, being relatively insoluble in water but soluble in organic solvents. The total lipid content of helminths is very variable, but is usually between 10 - 30% of the dry weight. Lipids have a variety of functions in tissues.

Lipid metabolism in cestodes has been worked out to only a limited extent. But gas chromatography and column chromatography has revolution lipid analysis. The role of lipids in cestodes metabolism is not clear, there is no evidence for example that lipid act as an energy reserves in cestodes as they do in nematodes. The synthesis of lipids is only studied in H. diminuta (Ginger and Fairbairn, 1966 a). In this species only a limited capacity for fatty acids biosynthesis has been demonstrated and most of its fatty acids appear to be desired from the host.

There is considerable variation in lipids from species from species and the degree of lipid content. Variation is also seen in the segments and regions of the worms being experimented, thus total lipid to be somewhat meaning less, unless the degree of maturity is known. The lipid content of some species grown in different hosts may vary substantially; H. diminuta (Ginger and Fairbairn, 1966 b) from Hammessten contained 9.5% lipid dry weight and those from long evens rate 16.5% dry weight Warren and Daughtery, 1957). In H. diminuta the lipids tend to be more abundant in the most posterior proglottids (Fairbairn Wetheim Harpur and Schiller, 1961). Figures for total thus tend to somewhat meaning less unless the degree of maturity is known.

In older proglottids the higher content of lipid has led to the view that much of this lipid largely represents waste products, of metabolism (Brand T. Von. 1952). One of the more unusual features of the composition of cestodes lipids in the fact that unsaponifiable material and phospholipids often account for more than 20% of the total lipids.
ESTIMATION OF LIPID

**Principle:**

Lipid contains a smaller proportion of oxygen than is present in the other types of food stuffs and therefore provide, per gram, more combustible carbon and hydrogen. The energy released in oxidation of one gram of fat is over twice that obtained from the same weight of either carbohydrate or protein. They are toughly divided into those which serve primarily as a store of oxidisable substance and those, which are part of the structural, like methanol, chloroform, there for their estimation.

**Reagents:**

1) Chloroform methanol: Mix 200ml of Chloroform in 100 ml of methanol (2:1).
2) Vanilline reagent: Dissolved 2gm of Vanilline in 200ml of distilled water and to it 800 ml of Orthophosphoric acid. (Keep it for one month to maturation) at least 15-20 days before use.
3) Blank: An aliquot of 0.2ml of Conc. H$_2$SO$_4$ + 5ml Vanilline reagent was taken in blank.
4) Standard stock solution: Dissolve 5mg of Cholesterol in 10ml of Chloroform: Methanol (2:1). Take 1ml of this solution and dry for two days at 370C. Add 1ml of conc. H$_2$SO$_4$ boil in water bath for 10 minutes and cool prepare 10 tubes for standard.
LIPID ESTIMATION IN
LYTOCESTUS Vyasaei n.sp.

MATERIAL AND METHODS:
The estimation of Lipid content in the cestode parasites was carried out by Barnes and Blackstock (1973) method.

Eleven Clarias batrachus fish were brought to the laboratory killed by pithing brain and dissected. The intestine carefully dissected and observed for collection of cestode parasites, four of them were found to be heavily infected with cestode parasites, with small pieces of infected and non-infected intestine was also collected to find out the lipid content of them these tissues.

The identical worms were separated and kept separately by observing them under microscope. Few of them were kept in 4% formalin for the identification. These worms later stained with Grenachers Borax Carmine and identified as Lytocestus vyasaei n.sp.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue was recorded. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then taken the dry weight of material, prepared a powder with the help of mortar pestle. The tissue is weighed 100 mgs on a sensitive balance and then it is homogenized in a mortar and pestle by adding 10 ml of 2:1 Chloroform: methanol solution and filter the mix by Whatman filter paper No. 41. The take pipette out 1 ml of filtrate and kept it for drying at 3-4 days at room temperature, then added 1 ml of concentrated H₂SO₄ and kept it in boiling water bath for 10 minutes, cool rapidly under water. The taken 0.2 ml of above solution and add 5 ml of Vanilline reagent, and this test tube was kept for half an hour at room temperature until the pink colour developed. Then this colour was read on the colorimeter with 530µm filter to note the optical density to calculate the lipid content.

For the standard of lipid, dissolve 5 mg of Cholesterol in 10ml of 2:1 Chloroform: Methanol. Take 1 ml of this solution and dry for two days at 37°C. Add 1 ml of concentrated H₂SO₄ boil in water bath for 10 minutes and cool. Then add 5 ml of Vanilline reagent and prepare the 10 test tube stock solution ranging from 0.01 to 0.1 ml and read the optical densities.
RESULT AND DISCUSSION

The Lipid content in *Lytocestus vyasaei* n.sp. and infected and uninfected intestinal tissues in the host *Clarias batrachus* was calculated by statistical analysis.

**Table -I.** Lipid content (mg/100mg of dry tissue) of *Lytocestus vyasaei* n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clarias batrachus</em> L.</td>
<td><em>Lytocestus vyasaei</em> n.sp.</td>
<td>5.27±1.27</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>2.27±0.13</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>3.41±0.17</td>
</tr>
</tbody>
</table>

The result represented that the lipid content of *Lytocestus vyasaei* n.sp. was higher than the infected and uninfected intestine, 5.27±1.27, 2.27±0.13 and 3.14±0.17 mg/100mg of dry wet tissue respectively.

The lipid content is more in the parasitic tissue as compared to their hosts. It seems that the parasites taking benefit from its host and is thus absorbing most of the nutrients. The parasite is fulfilling its needs from the host and it is in a way causing hindrance in the proper development of the host. This high level of lipid may also because, the parasite often absorbing the lipid stores from the further processes and lipid get exhausted very slowly.
LIPID ESTIMATION IN

**GANGESIA (GANGEISA) RAMKAEI** N.SP.

**MATERIAL AND METHODS:**

The estimation of Lipid content in the cestode parasites was carried out by Barnes and Blackstock (1973) method.

Eight *Wallago attu* fish were brought to the laboratory and dissected. The intestine carefully dissected and observed for collection of cestode parasites, five of them were found to be heavily infected with cestode parasites, with small pieces of infected and non-infected intestine was also collected to find out the lipid content of these tissues.

The identical worms were separated and kept separately by observing them under microscope. Few of them were kept in 4% formalin for the identification. These worms later stained with Grenachers Borax Carmine and identified as *Gangesia (Gangesia) ramkaei* n.sp.

The collected worms were dried on the blotting paper to remove excess of water and taken the wet weight of tissue was recorded. This material was transferred into previously weighed watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then taken the dry weight of material, prepared a powder with the help of mortar pestle. The tissue is weighed 100 mgs on a sensitive balance and then it is homogenized in a mortar and pestle by adding 10 ml of 2:1 Chloroform: methanol solution and filter the mix by Whatman filter paper No. 41. The take pipette out 1 ml of filtrate and kept it for drying at 3-4 days at room temperature then added 1 ml of concentrated H₂SO₄ and kept it in boiling water bath for 10 minutes, cool rapidly under water. The taken 0.2 ml of above solution and add 5 ml of Vanilline reagent, and this test tube was kept for half an hour at room temperature until the pink colour developed. Then this colour was read on the colorimeter with 530μm filter to note the optical density to calculate the lipid content.

For the standard of lipid, dissolve 5 mg of Cholesterol in 10ml of 2:1 Chloroform: Methanol. Take 1 ml of this solution and dry for two days at 37°C. Add 1 ml of concentrated H₂SO₄ boil in water bath for 10 minutes and cool. Then add 5 ml of Vanilline reagent and prepare the 10 test tube stock solution ranging from 0.01 to 0.1 ml and read the optical densities.
RESULT AND DISCUSSION:

The Lipid content in Gangesia (Gangesia) ramkaei n.sp. and infected and uninfected intestinal tissues in the host Wallago attu was calculated by statistical analysis.

Table I. Lipid content (mg/100mg of dry tissue) of Gangesia (G) ramkaei n.sp., infected and uninfected intestinal tissues.

<table>
<thead>
<tr>
<th>Host</th>
<th>Tissues</th>
<th>Lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallago attu</td>
<td>Gangesia (G.) ramkaei n.sp.</td>
<td>6.89±0.25</td>
</tr>
<tr>
<td></td>
<td>Infected tissue</td>
<td>5.57±0.25</td>
</tr>
<tr>
<td></td>
<td>Uninfected tissue</td>
<td>9.09±0.50</td>
</tr>
</tbody>
</table>

The result represented that the lipid content of uninfected intestine was higher (9.09±0.50) than the parasite and infected tissue but the infected tissue was less (5.57±0.25) than the Gangesia (Gangesia) ramkaei n.sp. (6.89±0.25).

In the present investigation different quantities of lipids are observed by the parasites, probably due to the difference in the amount of unsaturated fatty acids that can be permitted through the membrane system of parasites. The lipids content we can conclude that the percentage of lipid is more in the parasitic tissue as compared their host. The uninfected tissue of the same host are high content as compared of parasite tissue and infected tissue.
Figure no.15. Lipid content in *L. vyasaiei* n.sp., infected and uninfected intestinal tissues of *Clarias batrachus* (mg/100mg dry tissue).

Figure no.16. Lipid content in *G. (G) ramkaei* n.sp., infected and uninfected intestinal tissues of *Wallago attu* (mg/100mg dry tissue).
CONCLUSIONS:

The biochemical estimations of glycogen, protein and lipid from the cestode parasites, *Lytocestus vyasaei* and *Gangesia (Gangesia) ramkaei* collected from the host *Clarias batrachus* and *Wallago attu* and the infected and uninfected intestinal tissues of the host were came out. From the results and values it can be concluded that the endoparasites could be able to maintain the good balance in the glycogen, protein and lipid content with their hosts.

The glycogen and protein content is more in the cestode parasites as compared to the infected and uninfected intestinal tissue but the same was increased in uninfected (Healthy) intestinal tissue when compared with the infected intestinal tissue from both the hosts.

The lipid content of the *Clarias batrachus* uninfected intestinal tissue is slight more as compared to infected intestinal tissue and in the *Lytocestus vyasaei* n.sp. it was more as compared to the infected and uninfected intestinal tissue, where as in the host *Wallago attu* uninfected tissue was more as compared to infected and *Gangesia (Gangesia) ramkaei* but the infected tissue was less than the uninfected tissue.

The study of parasite physiology shows the existence of an intimate bond between the parasite and its environment. It is also major practical importance in preparing methods of sterilization to free meat and fish produce from larval helminthes, as well as in deciding on the treatment and prevention of parasitic diseases.

The site of the habitat and the mode of feeding of the parasite determine the type of metabolism. The most important factors determining the type of metabolism of cestodes is oxygen. Both oxy and anoxybionts show a clearly expressed Pasteur effect, but others display considerable stability of anoxybiotic metabolism even in the presence of oxygen. An important source of energy is glycogen, which is stored in the body of fish parasites in large quantities, determined by the stage of development. The fat is considered as the breakdown product of anoxybiotic metabolism. Its importance as a source of energy is not clear as yet. Both the parasites are absorbing the essential metabolic content from the host and are accumulated in the body of parasites, the high amount of glycogen, protein and lipid in the parasite shows that they absorb more from their macroclimate host body.
SUMMARY:

This chapter of this thesis deals with the biochemical estimations, like glycogen, protein and lipid of the cestode parasites, *Lytocestus vyasaei* n.sp and *Gangesia (Gangesia) ramkaei* n.sp from respective host, with infected and non-infected intestinal tissue of the host. The dry tissues were grounded to powder and utilized for estimation, glycogen was estimated by using Anthrone reagent as given by DeZwaan and Zandee (1972), using glucose as standard. Total lipids were estimated by using Vanillin reagent and Cholesterol as a standard as given by Barnes and Blackstock (1973) and the total amount of protein was estimated by Lowry’s method (Lowry’s et al., 1951) by using Folin and Ciocalteus phenol reagent and Bovine serum albumin as a standard.

The result indicate that the glycogen and protein content in infected intestinal tissue as compared to that of control host tissue (healthy host) was decreased in both host and in the *Lytocestus vyasaei* n.sp and *Gangesia (Gangesia) ramkaei* n.sp it was found increased.

The lipid content of the host *Clarias batrachus* infected intestinal tissue as compared to that of control was decreased and in the *Lytocestus vyasaei* n.sp was found increased, whereas in the host of *Wallago attu* infected intestinal tissue and *Gangesia (Gangesia) ramkaei* n.sp as compared to that of control it was seen decreased. The results are given in the table with percent changes over control (non-infected) and results of statistical test.