Part - III

BIO-CHEMISTRY
Introduction:

The gastrointestinal tract (GI) is not only an organ for digestion, absorption and excretion, but also it is a residence site to many parasitic organisms. The regulation of helminth population in the hosts GI is a complex process, influenced by host immunological and nutritional status, age and breed of the animal (Von Brand, 1979). Immunological status of the host is very important for helminth infections, because GI is one of the largest immunological organs of the body and it serves as the first line or defense against orally administered antigens (e.g. feed protein of carbohydrates) and intestinal pathogens. Gut associated lymphoid tissues make up about 25% by weight of the gut mucosa and sub mucosa and thus constitute the largest extrathymic sites of lymphocytes (McBurney, 1993). Furthermore, it is very important interaction between helminthes infection and nutrition. This interaction can be considered from two inter-related point of views (1) The adverse influence of the helminthes infection on the hosts physiology and nutrition (2) The effect of the host nutrition on the helminthes population i.e. their establishment, persistence and reproductive capacity (Coop and Holmes, 1996).

The first point of view (the impact of helminthes infection on the hosts physiology and nutrition) has been the subject of numerous investigations over the past decade (Stephenson, 1993; Solomons, 1993; Solomons & Scott. 1994; Eduisinghe & Tomkins, 1995; Coop & Holmes, 1996; Knox 2000). The research on the complex on the
complex interactions among host nutritional status and parasitic infection has mainly focused on the detrimental consequences of parasitic infections on host nutritional status and on mechanisms by which malnutrition impair immune-competence (Scott & Koski, 2000).

Nutritional deficiencies as a result of intestinal helminth infection have been the subject of serval investigations (Hadju, et al., 1996; Lunn & Nothrop- Clewer, 1996). Intestinal helminthes may affect the nutritional status by causing increased nutrient loss, in addition on decreased food intake and nutrient absorption (Edirishinghe & Tomkins, 1995). Detailed investigations of the mechanisms of gastrointestinal dysfunction of the parasitized host have shown that the increased endogenous loss of protein into the gastrointestinal tract is a key feature, partly as a result of leakage of plasma protein but also from increased exfoliation of gut epithelial cells and muco-protein secretion (Bown et al., 1991)

Curiously, the influence of host nutrition on helminthes population (the second type of host- parasite interaction) has received relatively little attention and limited information is available only a few studies have examined the effects of nutrition on the parasitic response in the parasited host, and even fewer have considered the event occurring at the intestinal level, where absorption of nutrients occurs, intestinal parasites reside, and the gastrointestinal associated tissues play role in directing both the local and the more systemic responses. Bundy & Golden (1987) described mechanisms by which host nutrition might influence helminthes infection: nutritionally mediated changes in the helminthes environment or nutritionally mediated changes in host
defense and malnutrition of the parasites. Gastrointestinal helminthes have very specific physio-chemical requirements of their host gut environment, and nutritionally mediated changes might have a direct influence on the parasite population (Crompton & Nesheim, 1976).

Parasitic worms compete for energy reserves with their fish host (Meakin, 1974). There is a clear variation among hosts and parasites in the extent of such effects. This may be due to difference in the extent to which the parasites compromises nutrient reserves.
PROTEIN

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 helminthes, protein usually constituents 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 protein 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 worm protein are very essential, therefore parasites do not depend on the host’s dietary protein. If host proteins are removed from the diet, even then 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 Nippostrogylus basiliensis proved some what unfavorable to the adult.
worm but indirect rather than direct influence was probably involved Clarke, (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 acid tries 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 of 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 others scientist (Good Child and Dennis 1965) did not get the same to all that due to the parasitic life led by Hymennolepis diminuta the composition of the amino acid pool and other parameters is changed (Mettrick, 1971) this can have an influence on the response of the parasites to an altered host diet. Further studies reveal that the molar ratio of amino acids differ with differing dietary proteins, as well as various pattern 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 oxidizes in Hymennolepis diminuta and some of changes in host, physiology by Daugherty (1955). Studies on protein complexes of the cestode Raillientina cesticillus by Kent (1957b).
Protein Estimation:

Principle: (Lowry’s Method)

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 burette 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) Reagents:

1. **Lowry’s ‘A’ solution**: Dissolve 2gms of Na2Co3 in 100ml of 0.1N NaoH solution (400mg of NaoH in 100 ml of distilled water = 0.1 N NaoH)

2. **Lowry’s ‘B’ solution**: B1 = 1% Copper Sulphate (CuSo4), B2 = 2% Potassium Sodium taritrate

3. **Lowry’s ‘C’ solution**: 1ml of B1 + 2ml of B2 diluted to 100ml with Lowry’s ‘A’ solution

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

5. **1 N NaoH**: Dissolved 4gm of NaoH (Sodium Hydroxide) in 100 ml of distilled water.
6. **Folin Phenol reagent**: This reagent is diluted by distilled water in preparation of 1:1.

7. **Standard stock solution**: 10mg of BSA (Bovine Serum Albumin) in 10ml of 1 N NaOH.

8. **Blank solution**: 0.1 ml (1 N NaOH) + 4ml of Lowry’s ‘C’ solution + 0.4ml of Folin Phenol.

**Material and Method (Lowery's method)**:

The collected worms were dried on the blotting paper to remove excess of water and the wet weight of tissue was recorded. This material was transferred into previously weight watch glass and kept in oven at 58 to 60°C for twenty-four hours, for drying and then the dry weight of material, prepared a powder with the help of mortar pestle. The powder weight 100.00mg 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 3000RPM. Supernatant is discarded and residue is taken and added 10ml of N NaOH for dissolving the ppt. Then taken 0.1 ml above solution and add 4 ml Lowery's ‘C’ solution, then it add 0.4ml 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 spectrophotometer with 660nm wavelength, filter no 4. 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 observation:**

Mean values of Protein content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Cestode parasites</th>
<th>Protein Content (mg/gm wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parasites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>26.57</td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>24.85</td>
</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>26.80</td>
</tr>
<tr>
<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>26.75</td>
</tr>
<tr>
<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed).</td>
<td>25.52</td>
</tr>
<tr>
<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed).</td>
<td>20.73</td>
</tr>
<tr>
<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed).</td>
<td>16.25</td>
</tr>
<tr>
<td>8</td>
<td><em>Raillietina (R) quadriest testiculata</em>, Moghe, 1925 (Redescribed)</td>
<td>20.25</td>
</tr>
</tbody>
</table>
Graph shows mean values of Protein content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

**Graph - 1**

**Graph - 2**
Result and Observation:

The protein is estimated by folin phenol reaction by Lowery's et. al. (1951) in different species of Cotugnia and Raillietina from Gallus gallus domesticus. Table No.1 and graph G-1 data showing protein content of the worm, reveal that there is little variation in content.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 24.85 (mg/gm wet wt) in summer season and highest being 26.57 (mg/gm wet wt) in winter season.

*Cotugnia hyderabadensis* sp. Nov. The lowest being 23.05 (mg/gm wet wt) in summer season and highest being 24.85 (mg/gm wet wt) in winter season.

*Cotugnia pratibhae* sp. Nov. The lowest being 24.05 (mg/gm wet wt) in the summer season and highest being 26.80 (mg/gm wet wt) in winter season.

*Cotugnia govindi* sp. Nov. The lowest being 24.65 (mg/gm wet wt) in summer season and highest being 26.75 (mg/gm wet wt) in winter season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). The lowest being 23.15 (mg/gm wet wt) in summer season and highest being 25.52 (mg/gm wet wt) in winter season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed). The lowest being 18.98 (mg/gm wet wt) in summer season and highest being 20.73 (mg/gm wet wt) in winter season.
Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed). The lowest being 15.17 (mg/gm wet wt) in summer season and highest being 16.25 (mg/gm wet wt) in winter season.

Raillietina (R) quadristesticulata, Moghe, 1925 (Redescribed) The lowest being 18.21 (mg/gm wet wt) in summer season and highest being 20.25 (mg/gm wet wt) in winter season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 17.42 (mg/gm wet wt) in summer season and highest being 19.42 (mg/gm wet wt) in winter season.

The folin-phenol reaction described by Lowery's et. al. (1951) is generally used for the extraction of protein, when protein is found in micro quantities. The values obtained by this procedure in various parasitic helminthes were found to vary. The species specific difference was recorded in various helminth parasites. The quantities of protein Cittotoina perplexa 21%, (Campell, 1960); E. granulossus 61% (Agoisn et. al., 1957); M. expansa 22% (Campell,1960); R. cesticillus 36% (Reid,1942); T. taenaeformis larva 27 to 29% and adult 45% (Von Brand and Bowman). Halberg (1973) classified factors influencing individual biochemical variability as interindiviuval and intraindividul.
**GLYCOGEN**

**Introduction:**

The carbohydrate, which include low molecular weight (LMW) sugar and various cell wall and storage non-starch polysaccharides (NSP) are the most important energy sources for animal (Bach Knudsen, 1997). It is now clear that dietary carbohydrates are diverse group of substances with varied fares in the gastro-intestinal tract and physiological properties of differing important to animal health (Cummings & Englyst, 1995). The composition of the carbohydrate fraction influences the digestion and absorption process of carbohydrate and other nutrients in various parts of the gastro-intestinal tract. It has profound influence on the secretary response of the gut to feed intake (Low, 1989), the volume flow (Bach Knudsen et al., 1993), the mucosal architecture (Brunsyaard, 1998), composition of the gut flora (Jensen & Jorgensen, 1994) and the development of the gastrointestinal tract (Jorgensen et al., 1996). Studies on the influence of carbohydrates on growth of parasite and establishment have been limited mainly to cestode and acanthocephalan (Crompton & Nesheim, 1982; Nesheim, 1984).

The main carbohydrate reserve in parasitic helminth is “Glycogen” which is a typical energy reserve of helminthes inhabiting biotopes with low oxygen tension. The main polysaccharide in cestode is glycogen, closely resembling mammalian glycogen. The early work of Foster (1856) demonstrated the occurrence of glycogen in helminthes.
Glucose is said to be an important energy source for helminthes inhabiting the alimentary tract of vertebrates. It is generally believed that helminthes absorbed 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) have pointed out that the cestode have high rate of transport of exogenous glucose into the body, high rate of utilization of endogenous carbohydrate and high rate of glycogenesis, the cestode parasites as a group store relatively large quantities of polysaccharides, which is most cases have assumed to be glycogen, Reid (1942).

The literature at our disposal discloses that the carbohydrates play an important role in cestode, than most of other parasitic worms, which are distinguished by different growth patterns. These carbohydrates are utilized exogenously, their mechanism of the up take is not known but the evidence indicates that the active mechanism undoubtedly is entangled in the carbohydrate transport of helminthes. In cestode *Hymenolepis diminuta* the absence or restriction of availability of dietary carbohydrates resulted in decreased establishment, growth and reproduction (Robert, 1980). Dunkley and Metrick (1969) have found that in rat, fed by sucrose containing diet were found smaller *Hymenolepis diminuta* worms than in rats on glucose or maltose diets. Additionally, it was found that *Hymenolepis diminuta* worms from high starch diet rat were bigger than low starch rats, which are bigger than from sucrose diet rats (Roberts, 1966), Caliobothrium vertiallatum (Fisher, 1965) as well as the Acanthocephalan Polymorphus minutus.
(Crompton and Lackwood, 1968) absorb glucose against concentration gradients and the survival, growth and reproduction of Moniliformis moniliformis are dependent on the carbohydrates liberated at different rates from the intestinal tract of the host during digestion and absorption (Nesheim et al., 1977, 1978). Further more typical inhibitors of active transport e.g. philoridizin interferes effective with the glucose uptake of cestodes (Phifer, 1960a). 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 hrs and then slows down, even if the concentration is very high as it was the early phase. It has been observed the same in *Hymenolepis diminuta* increase from 15% of the dry substance in 5 and 7 day old worms to 37% in 13 and 16 days old specimens (Mettric and Cannon, 1970), it has been observed that the uptake of glucose is very much effective when Co2 is present in the surrounding than it is absent.

A number of workers have reported the content of glycogen in different helminth parasites. In *Hymenolepis diminuta* from 1.1 to 9.3% (Fairbairn et al., 1961) 16% in Schistocephalus solidus larva (Hopkins, 1950) respectively.
Estimation Of Glycogen (DeZwaan and Zandee (1972) Method):

Principle:

The Anthrone reaction is a 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: Dissolved 30gms Of KOH (Potassium Hydroxide) In 100ml Of Distilled Water.
2. 2% Na2SO4 Solution: Dissolved 2 gms of Sodium Sulphate (Na2SO4) in 100ml of distilled water.
3. 100% ethyl solution.
4. Anthrone reagent: Dissolved 150mg of Anthrone in 100ml of concentrated Na2SO4 (88%)
5. Standard stock solution: 100 mg of glucose in 100ml distilled water.

Material and Method:

Glycogen Estimation DeZwaan and Zandee (1972) method:

The collected worms were dried on the blotting paper to remove excess of water and transferred in a previously weight watch glass, weighted on a sensitive balance.
The wet weight of the tissue taken and kept in oven at 58 to 60°C for twenty-four hours to make material dry. The dry weight of the material was taken and prepared the powder. The powder was weighted 100mg on a sensitive balance and was homogenized by mortal pestle adding 1 ml of 30% KOH to it and transferred in centrifuge tube kept in boiling water bath for 3 to 5 minutes, cooled at room temperature, then adds 0.2ml of 2% Na2SO4 solution. Add to the same 6ml 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 10ml of distilled water bath for 10 minutes and immediately cooled and reading were taken with 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.

**Glycogen Estimation:**

Fifteen collected cestodes and small pieces of infected and intestines were also collected to find the Glycogen content in them using DeZwaan and Zandee (1972) method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for identification.
Mean values of glycogen content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus* / *Capra hircus*.

Table No. 2

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Cestode parasites</th>
<th>Glycogen content (mg/gm wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Winter</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>21.15</td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>15.85</td>
</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>20.03</td>
</tr>
<tr>
<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed)</td>
<td>18.97</td>
</tr>
<tr>
<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed)</td>
<td>18.11</td>
</tr>
<tr>
<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed)</td>
<td>16.19</td>
</tr>
<tr>
<td>8</td>
<td><em>Raillietina (R) quadristerculata</em>, Moghe, 1925 (Redescribed)</td>
<td>19.08</td>
</tr>
</tbody>
</table>
Graph shows mean values of glycogen content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

Graph No. 2
Results and observation:

The glycogen is estimated by DeZwaan and Zandee (1972) method in different species of *Cotugnia* from *Gallus gallus domesticus*. Table No.2 and graph G-2 data showing glycogen content of the worm, reveal that there is little variation in content.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 20.93 (mg/gm wet wt) in rainy season and highest being 21.62 (mg/gm wet wt) in summer season.

*Cotugnia hyderabadensis* sp. Nov. The lowest being 14.74 (mg/gm wet wt) in rainy season and highest being 16.21 (mg/gm wet wt) in summer season.

*Cotugnia pratibhae* sp. Nov. The lowest being 19.98 (mg/gm wet wt) in rainy season and highest being 21.87 (mg/gm wet wt) in summer season.

*Cotugnia goindi* sp. Nov. The lowest being 12.89 (mg/gm wet wt) in rainy season and highest being 14.41 (mg/gm wet wt) in summer season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). The lowest being 17.07 (mg/gm wet wt) in summer season and highest being 19.36 (mg/gm wet wt) in winter season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed). The lowest being 18.98 (mg/gm wet wt) in summer season and highest being 18.97 (mg/gm wet wt) in winter season.
Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed). The lowest being 15.17 (mg/gm wet wt) in summer season and highest being 16.25 (mg/gm wet wt) in winter season.

Raillietina (R) quadristesticulata, Moghe, 1925 (Redescribed) The lowest being 17.84 (mg/gm wet wt) in rainy season and highest being 19.64 (mg/gm wet wt) in summer season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 18.04 (mg/gm wet wt) in rainy season and highest being 19.15 (mg/gm wet wt) in summer season.

From the table No. 3 and graph G-3 showing the lipid content highest being 29.62 (mg/gm wet wt) Cotugnia shrirampurensis sp. Nov. in summer season and lowest being Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed) 14.08 (mg/gm wet wt) in rainy season.

The glycogen content shows marked variation within any species in relation to the nutrition of the host and the degree of maturity of the strobila, especially intestinal parasites, thus in Raillietina cesticillus, the glycogen content decreases from 4.6% to .25% (wet weight) when the host is starved for 20 hours (Read, 1942). According to (Lee et. al., 1970) glycogen disappears rapidly from the tissues of the nematodes when the host is starved.

Environment also has great influence of glycogen parasites inhabiting low oxygen content environment have high glycogen content (Von Brand, 1973), it is presumed that these variation occur due to hormonal control of the host.
From the above evidence it is clear that the glycogen content was influence by the nutrition of the host, maturity of the strobila, starvation and environmental factors.

Brand von (1973) suggested that the tapeworm obtain their nutrient supplu either from intestinal mucosa or exocrine- interic circulation of the host. Hence it is considered view of the author that the observed fall result in glycogen content in the infected host tissue might have occurs due to physiological dysfunction in the intestinal tissue of host which there by into decrease rate of glucose supply to the intestine.

The glycogen reserve is in fact a sensitive indicator of variations in metabolic conditions is well shown by the experiments of Brand (1933) and Reid (1942) the former found tha in Taenia marginata the glycogen content was increased from 4.99% to 8.38% when the host dog was fed on a carbohydrate- rich diet. Conversely Reid found the glycogen reserved fell by over 90 per cent in Raillietina cesticillus after 20 hours starvation of the host fowl.
ESTIMATION OF LIPID

Introduction:

Cestodes are incapable of de Novo synthesis of nonvolatile saturated and unsaturated fatty acids (Meyer et al., 1966) and have been shown to rely upon their hosts to supply fatty acids for lipid biosynthesis. Thus, the fatty acids of these worms reflect to varying degrees or host intestinal contents (Ginger and Fairbairn, 1966a).

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

There is a considerable variation in lipids from species to species, in the degree of lipid content. Variation is also seen in the segments and region of the worms being experimented, thus total lipid to be some what meaning less, unless the degree of maturity is known. The lipid content of some species grown in different hosts may vary substantially. In *Hymenolepis diminuta* the tend to be more abundant in the most posterior proglottids (Feirbairn Wetheim, Harpur and Schiller, 1961).

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 cestode lipid in the fact that unsaponifiable material and phospholipids often account for more than 20% of the total lipids.
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. Then 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 therefore they are estimated.

Reagents:

1. Folch’s mixture  Chloroform 2 Parts : Methanol 1Parts
2. Vanilline reagent: Dissolve 1225 mg Vanilline in 125 ml of distilled water. Add 500 ml of Orthophosphoric acid. Keep in dark bottle for ripening for one month.

Material and Method:

Barnes and Blackstock (1973) method:

The collected worms were dried on the blotting paper to remove excess water and wet weight of tissue was recorded. This material transferred into previously weight watch glass and kept in oven at 58 to 60°C for twenty hours for drying. The dried weight of the material was recorded and powder was the help of mortar pestle, 100mgs of the tissue was weighted on a sensitive balance, homogenized in a mortar and pestle by adding 10 ml of 2:1 chloroform: methanol solution, filter the mixture by Whatman filter paper No.41. The 1 ml of filtrate
was pipette out, dried for 3-4 days at room temperature, then 1ml of concentrated H2SO4 was added and kept in boiling water bath for 10 minutes and cooled rapidly under water, 0.2 ml of above solution is added in 5ml of vanilline reagent and the test tube were kept for half an hour at room temperature until the pink colour develop. The optical density was calculated by using calorimeter with 530µm filter to note the lipid content.

For the standard of lipid, 5mg cholesterol is added in 100ml of 2:1 chloroform: methanol. Take 1ml of this solution and dried for two days at 37°C, Then 1ml of concentrated H2SO4 boil in water bath for 10 min and cool. Then add 5ml of vanilline reagent and prepare the 10 test tube stock solution from 0.01 to 0.1 ml and read the optical densities.

Fifteen collected cestodes and small pieces of infected and intestines were also collected to find the Lipid content in them using Barnes and Blackstock (1973) method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formlin for identification. These were later stained with Harris Haematoxylin and identified.
Mean values of lipid content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

Table No. 3

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Cestode parasites</th>
<th>lipid content(mg/gm wet wt)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>parasites</td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shirampurensis</em> sp. Nov.</td>
<td>29.15</td>
<td>29.62</td>
<td>28.36</td>
<td>30.67</td>
<td>31.20</td>
<td>29.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>24.95</td>
<td>25.40</td>
<td>24.19</td>
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<td>28.11</td>
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<td>26.26</td>
<td>26.36</td>
<td>24.15</td>
<td>32.47</td>
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<td>31.13</td>
<td></td>
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<tr>
<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>25.86</td>
<td>26.40</td>
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<td>28.09</td>
<td>30.20</td>
<td>26.69</td>
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<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed).</td>
<td>17.64</td>
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<td>28.46</td>
<td>28.32</td>
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<td>7</td>
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<td>15.13</td>
<td>15.37</td>
<td>14.08</td>
<td>28.79</td>
<td>29.36</td>
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<td>8</td>
<td><em>Raillietina (R) quadristesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>16.05</td>
<td>16.26</td>
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<td>30.52</td>
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</table>
Graph shows mean values of lipid content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

**Graph 3**

[Graph showing lipid content for different parasites and intestinal tissue in different seasons]
Results and obsevation:

The lipid content is estimated by Barnes and Blackstock (1973) method in different species of *Cotugnia* and *Raillietina* from *Gallus gallus domesticus*. Table No.3 and graph G-3 data showing lipid content of the worm, reveal that there is little variation in lipid content.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 28.36 (mg/gm wet wt) in the rainy season and highest being 29.62 (mg/gm wet wt) summer season.

*Cotugnia hyderabadensis* sp. Nov. The lowest being 24.19 (mg/gm wet wt) in the rainy season and highest being 25.40 (mg/gm wet wt) summer season.

*Cotugnia pratibhae* sp. Nov. The lowest being 24.15 (mg/gm wet wt) in rainy season and highest being 26.36 (mg/gm wet wt) in summer season.

*Cotugnia govindi* sp. Nov. The lowest being 18.12 (mg/gm wet wt) in rainy season and highest being 26.40 (mg/gm wet wt) in summer season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). The lowest being 25.77 (mg/gm wet wt) in rainy season and highest being 28.64 (mg/gm wet wt) in summer season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed). The lowest being 16.95 (mg/gm wet wt) in the rainy season and highest being 18.77 (mg/gm wet wt) in summer season.
Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed). The lowest being 14.08 (mg/gm wet wt) in rainy season and highest being 15.37 (mg/gm wet wt) in summer season.

Raillietina (R) quadritesticulata, Moghe, 1925 (Redescribed) The lowest being 15.21 (mg/gm wet wt) in rainy season and highest being 16.26 (mg/gm wet wt) in summer season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 16.18 (mg/gm wet wt) in summer season and highest being 19.4218.24mg/gm wet wt.) summer season.

From the table No. 3 and graph G-3 showing the lipid content highest being 29.62 (mg/gm wet wt.) Cotugnia shrirampurensis sp. Nov. in summer season and lowest being Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed) 14.08 (mg/gm wet wt) in rainy season.

From the present investigation it appears that lipids content more or less remains static, it shows only minor fluctuations, Fair brain et.al. (1961) and Gretchus and Greichus (1966, 67). the lipid content of same species in different hosts may very substantially, H. diminuta from hamsters contained 9.5% lipid (dry weight) and those from long-Evants rats 16.5% (dry weight) (Warren and Daugherry, 1957).

Nigam and Premavati (1980) worked on the effect of host diet on fatty acid composition of C. digonopora and R. fuhrmanni, the other cestodes which have been worked for lipids are R. echinobothrium, M. expansa, T. saginata, D. latum and E. granulous. Lipid percentage varies according to seasons in Stilesia leiperi from Ovis bharal was worked out by Jaghav et. al. (1980). it is clear that the lipid content was changed by the host diet and the effect of climatic factors.
PYRUVATE

Introduction:

Glucose is broken down by glycolysis as far as phosphoenol pyruvate (PEP) stage this PEP is converted to pyruvate by the action of pyruvate kinase and then reduced to lactate by Lactate dehydrogenase and NADH, mn++ and k+ are essential for the activity for the pyruvate kinase.

Pyruvate acid plays an important role in the energy metabolism of parasitic helminthes. It is formed by the action of pyruvate kinase is the presence of mg++. It is the end product of glycolysis under aerobic conditions and is converted into the acetyl coenzyme-A by oxidative decarboxylation. This reaction catalysed by a complex of multiple enzymes designated as the pyruvate dehydrogenase complex. The acetyl coenzyme-A thus formed is the starting compound of the Kreb’s cycle.

The carbohydrate, protein and lipid metabolism also meet at the pyruvic acid formation stage. Further it is known that some of the protein intermediates such as glutamic acid, aspartic acid and pyruvic acid respectively (Barrett, 1982).

Schiebel and Saz (1996) have shown another method of formation of pyruvate without the intervention of pyruvate kinase. They have shown that phosphoenolpyruvate is converted into oxaloacetate due to the fixing of CO2 (Saz and Lescure, 1969). The oxaloacetate is reduced to malate by the action of malate dehydrogenase (Bueding and Saz, 1968). The malates enter the mitochondria and undergoes dismutation. One half of the malate is decarboxylated by malic enzyme to form pyruvate (Saz
and Hubbard, 1957, Saz and Lescure, 1969, Agosin and Repetto, 1965). The pyruvate so formed is probably the precursor of acetate (Saz, 1970).

Pyruvate also serves as substrate for transamination and help in the formation of amino acid from the carbohydrates. In Echinococcus granulossus in which it has been shown (Agosin and Repetto, 1963) that a number of kreb’s cycle intermediate were oxidized not only in homogenation but also in intact protoscoleces. Incomplete evidence is available for the existence of Kreb’s cycle in other genera such as Tania, Moniezia and Hymenolepis, (Read, 1968). Small quantities of pyruvate and lactate are produced under aerobic conditions by protoscoleces of *Echinococcus granulosus* in anaerobic conditions. Pyruvate is absent and more lactate is found (Agosin, 1957).

Several species of pathogenic Trypanosomes of Africa (Reley, 1956) metabolise glucose and transform almost quantitatively into pyruvate and recreated only traces of lactate. The pyruvate so formed by the Trypanosome is excreted out of their bodies and utilized by the host for its metabolic needs (Von Brand, 1973).

A survey of literature shows that not much work has been carried out on the pyruvate content in cestode parasites with their host though it occupies a very important position in the energy metabolism. Therefore in the present investigation pyruvate content in the intestinal tissue of *Gallus, gallus domesticus* infected by cestode. In contagia and in *Raillietina* pyruvate content in the different body region has been estimated quantitatively.
**Principle:**

The deproteinated filtrate when treated with 2.4 Dinitrophenyl hydrazine forms a 2.4-Dinitrophenyl hydrazine, which reacts with a strong alkali other keto acids reacts to form hydrazone con preferentially be extracted with sodium carbonate which is estimated photometrically by the method of friedmann and Haughen (1943) as described by Hawk (1954)

**Material and Method:**

The worm were weighted and homogenized in cold glass distilled water. Each volume of 10% Trichloro acetic acid (TCA) was added to precipitate proteins. The homogenates was then centrifuged for 15 min at 2500 rpm. The sediment which free of protein was used for the estimation of pyruvic acid by friedmann and Haughen (1943) method

**Reagents required:**

1) **Standard pyruvic acid solution**: 107 mg solution pyruvate was dissolved in 100 ml of 0.1 N sulphyric acid. The working standard was prepared by diluting 1ml of the standard solution to 100 ml disklled water. This standard solution contains 0.01 mg pyruvic acid per ml.

2) **10 % sodium carbonate**: 10 mg of sodium carbonate was dissolved in 100 ml of glass distilled water.

3) **10 % Trichloroacetic acid**: 10 mg of TCA was dissolved in 100 ml of glass distilled water.

4) **1.5 N sodium hydioxide**: 6 mg of sodium hydroxide was dissolved in 100 ml of glass distilled water.
5) **2.4-Dinitrophenyl hydrazine:** 100 mg of 2.4- Dinitrophenyl hydrazine was dissolved by adding small volume of 2 NHCl and made up to 100 ml. the content were then filtered was stored in cold condition.

**Procedure:**

To 3ml of protein – free filtrate, 1 ml of 2.4- Dinitrophenyl hydrazine was added and allowed to react for about 5 ml at room temperature 3 ml of working standard solution and reagent blank ( 3 ml of working standard solution) and reagent blank ( 3 ml diskilled water) were taken in two separate test tubes. To all the test tube.3 ml of foluene was added. The content were mixed by passing a stream of air for 3 minutes and then allowed to settle. The lower layer was discarded with a capillary tip droper. 6ml of 10% sodium carbonate was added to each test tube and content were once again mixed by passing air for 2 min. The mixture was then allowed to settle 5 ml of the lower layer of each tube was transferred into fresh test tube and 5ml of 1.5 N sodium hydroxide was added. The test tube were left for 10 minutes and then the optical density of the test sample was read against the blank at 546hm in spectrophotometer .

**Calculation:**

- \( \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \text{conc. of standard} \)
- Amount of pyruvic acid in sample.

- The pyruvate content of fresh tissue was calculated from this as µg of pyruvatel gm.
Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formlin for identification. These were later stained with Harris Haematoxylin and identified.
Mean values of pyruvate content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

Table 4

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Cestode parasites</th>
<th>Pyruvate content(µgm/gm fresh wt)</th>
<th></th>
<th></th>
<th>Winter</th>
<th>Summer</th>
<th>Rainy</th>
<th>Winter</th>
<th>Summer</th>
<th>Rainy</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>parasites</td>
<td>intestinal tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
<td></td>
<td></td>
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<td>38.76</td>
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<td>36.71</td>
<td>54.26</td>
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<td>53.64</td>
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<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
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<td>39.75</td>
<td>50.84</td>
<td>52.18</td>
<td>49.27</td>
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<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
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<td>49.54</td>
<td>38.36</td>
<td>57.17</td>
<td>58.20</td>
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<td><em>Cotugnia govindi</em> sp. Nov.</td>
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<td>49.84</td>
<td>42.26</td>
<td>56.93</td>
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<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed).</td>
<td>33.36</td>
<td>48.45</td>
<td>38.28</td>
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<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed).</td>
<td>44.32</td>
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<td>51.47</td>
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<td>8</td>
<td><em>Raillietina (R) quadritesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>38.13</td>
<td>43.65</td>
<td>39.79</td>
<td>47.07</td>
<td>49.36</td>
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<td>9</td>
<td><em>Raillietina (R) friedbergeri</em>, (Linstow, 1978), Fuhrmann, 1920 (Redescribed).</td>
<td>36.45</td>
<td>42.66</td>
<td>37.09</td>
<td>47.43</td>
<td>48.68</td>
<td>45.56</td>
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</tbody>
</table>
Graph shows Mean values of pyruvate content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

Graph - 4
Result and observation:

Data showing pyruvate content of the worm, reveal that there is little variation in the contents of Cotugnia and Raillietina species in the different season of the year.

*Cotugnia shrirampurensis* sp. Nov. the lowest being 36.71 (µgm/gm fresh wt) in the rainy season and highest being 47.67 (µgm/gm fresh wt) summer season.

*Cotugnia hyderabadensis* sp. Nov. The lowest being 39.75 (µgm/gm fresh wt) in the rainy season and highest being 47.54 (µgm/gm fresh wt) summer season.

*Cotugnia pratibhae* sp. Nov. the lowest being 37.56 (µgm/gm fresh wt) in winter season and highest being 49.54 (µgm/gm fresh wt) in summer season.

*Cotugnia goindi* sp. Nov. the lowest being 42.26 (µgm/gm fresh wt) in rainy season and highest being 49.84 (µgm/gm fresh wt) in summer season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). the lowest being 33.36 (µgm/gm fresh wt) in winter season and highest being 248.45 (µgm/gm fresh wt) in summer season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed). The lowest being 39.42 (µgm/gm fresh wt) in rainy season and highest being 45.47 (µgm/gm fresh wt) in winter season.
*Raillietina (R) fuhrmanni*, Southwell, 1922 (Redescribed). The lowest being 40.56 (µgm/gm fresh wt) in rainy season and highest being 44.32 (µgm/gm fresh wt) in the winter season.

*Raillietina (R) quadristesticulata*, Moghe, 1925 (Redescribed) the lowest being 38.13 (µgm/gm fresh wt) in winter season and highest being 43.65 (µgm/gm fresh wt) in summer season.

*Raillietina (R) friedbergeri*, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 36.45 (µgm/gm fresh wt) in summer season and highest being 42.66 (µgm/gm fresh wt) summer season.

From the table No. 4 and graph - 4 showing the pyruvate content highest being 49.84 (µgm/gm fresh wt) *Cotugnia govindi* sp. Nov. in summer season and lowest being 33.36 (µgm/gm fresh wt) *Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). In winter season.
LACTATE

Introduction:

It was supposed earlier that lactate is not the major end product of glycolysis in helminth parasites. A closer study of key metabolites and enzymes of glycolysis in the present study showed invariably the presence of lactate in the parasites contrary to the expectation. Lactic acid is typical end product of glycolysis. The reduction of pyruvate to lactate to lactate involves the reoxidation of reduced to factor NADH produces by glyceraldehydes 3 phosphate dehydrogenase into NAD which help in preventing the glycolysis from coming to an end. In vertebrate the cactate produced forms pyruvate in anaerobic conditions in aerobic phase. Pyruvate is formed and is incorporated into the kreb’s cycle. In helminth parasites lactate is excreted under anaerobic condition without being further metabolized.

Fairbairn (1961) suggested that, the excretion of lactic acid by a number of small helminth parasites may be related to the surface volume ration of those parasites. In large parasites lactic acid has not been reported tobe a major product of fermentation fairbairn (1961) observed that when 6 days and 14 days larvae of Hymenolepis diminuta were maintained in vitro, the 6 days, larvae secreted lactic acid in relatively large amount than succinic acid acetic acids, whereas in 14 days old larvae excretion of succinic acid and acetic acid was greater than that of lactic acid. Hence, the shift during development of patrasites is from lactic acid to succinic acid.
Laurie (1957) observed lactate to be the major end product of *Hymenolepis diminuta*. In *Echinococcus granulossus* larval forms, the major end products were found to be the lactate (Agosin, 1957). In the anaerobic conditions more lactate was produced and pyruvate does not exist, whereas in aerobic conditions trace of acetic, pyruvic and succinic acid were found, Bryant and Bhem (1976) have shown that under both aerobic and anaerobic conditions lactate was found in *Moniezia expansa*.

According to (Smyth 1969) the production of lactate differs from species to species and between adult and larval forms of same species. Further he also studied the quantity of end product by the parasites vary with the gas phase which they are studied and also in different physiological conditions of the host (Starved and Refeed). In *Hymenolepis taeniformis* (Von Brand and Bowman, 1961) 23% of total end product under aerobic phase was lactic acid whereas 15% in aerobic phase. Cestode excretes a number of volatile fatty acids, including acetate, prosponate and butyrate. Ethanol is an end product of carbohydrate, catabolism in some parasites for example larval scolices of *Echinococcus granulous larval* and adult forms of *Taenia taeniformis* and *Monoliformis dubis*. The production of lactate or succinate, end product is indicative of enzymatic activity pyruvate kinase and phosphoenol pyruvate carboxy kinase. The production of lactate would give a lower yield of energy molecule of glucose, a greater flow of carbon along the metabolic pathway has to be necessarily accomplished (Bryant, 1976).
Lactate (method):

The worm were weighted and then homogenied in 10% Trichloroacetic acid. The homotenates was the centrifuged at 3000 rpm for 15 minutes. The filtrate was used for the estimation of lactic acid by the modified method of (Hollanders, 1961)

Reagents required:

1. **10% TCA**: 10mg of TCA was dissolved in 100 ml of double distilled water.

2. **20 % copper sulphate solution**: 20 mg of copper sulphate was dissolved in 100 ml of double distilled water.

3. **4 % copper sulphate solution**: 4 mg of copper sulphate was dissolved in 100 ml of distilled water.

4. **0.1% parahydroxyl diphenyl reagent**: 100 mg of parahydroxyl diphenyl was dissolved in a little of 5% sodium hydroxide solution and then made up to 100 ml by double distilled water.

5. **Lithium lactate stock standard solution**: 0.213 gm of dry Lithium lactate was dissolved in 100ml of distilled water in one liter volumetric flask. 1 ml of conc.sulphuric acid was added to it, and then diluted with distilled water up to mark. It is stable indefinitely when kept in refrigerator (5ml of this solution contains) ml of lactates.

6. **Working standard solution**: 5ml of stock standard solution was made up to 100ml by adding double distilled water 1ml of this working standard solution contains 0.01mg of lactic acid. Tobe
prepared freshly every time. Procedure 5ml of Lithium lactate standard solution (0.01 mg) 2ml of deproteined filtrate and a reagent blank (2ml of distilled water)

Were taken in three separate gradated centrifuged tubes. To all the centrifuge tubes, 1ml of 20% copper soluphate solutions was added and then made up to 10ml by adding double distilled water. To each centrifuge tube 1 gm of calcium hydroxide powder was added and left for an half an hour with repeated shaking. The tubes were then centrifuged at 2500 rpm for 25 minutes from each tube 1 ml of supernatant was transferred into a otean dry test tube and 6ml of chilled annular sulphuric acid was added. The tubes were then placed in a hot water bath for 5 minutes and cooled at 20ºc Two drops of 4% copper sulphate solution and 0.5 mlof parahydroxy diphenyl reagent was added to all the tubes and then shaken thoroughly. The tube were then incubated for 30 minutes at 30I1ºc later boiled for go seconds and then allowed to cool room temperature. The optical density was read at 720nm against blank in spectrophotometer. The values are expressed as mg lactale 1gm fresh weight of the parasite

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out, few of these were fixed in 4% formlin for identification. These were later stained with Harris Haematoxylin and identified
Mean values of lactate content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

Table No. 5

<table>
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<th>Sr. No</th>
<th>Cestode parasites</th>
<th>lactate content (mg/gm fresh wt)</th>
<th>parasites</th>
<th>intestinal tissue</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>0.56</td>
<td>0.65</td>
<td>0.55</td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>0.68</td>
<td>0.79</td>
<td>0.69</td>
</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>0.42</td>
<td>0.53</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>0.76</td>
<td>0.88</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed)</td>
<td>0.86</td>
<td>0.95</td>
<td>0.76</td>
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<tr>
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<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed)</td>
<td>0.76</td>
<td>0.85</td>
<td>0.78</td>
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<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed)</td>
<td>0.65</td>
<td>1.03</td>
<td>0.86</td>
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<td>8</td>
<td><em>Raillietina (R) quadristesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>0.88</td>
<td>0.98</td>
<td>0.81</td>
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<tr>
<td>9</td>
<td><em>Raillietina (R) friedbergeri</em>, (Linstow, 1978), Fuhrmann, 1920 (Redescribed)</td>
<td>0.89</td>
<td>1.01</td>
<td>0.79</td>
</tr>
</tbody>
</table>
Graph shows Mean values of lactate content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

**Graph - 5**
Result and observation:

Data showing lactate content of the worm reveal that there is little variation in the contents of *Cotugnia* and *Raillietina* species in the different season of the year.

*Cotugnia shrirampurensis* sp. Nov. the lowest being 0.55 (mg/gm wet wt) in rainy season and highest being 0.65 (mg/gm wet wt) summer season.

*Cotugnia hyderabadensis* sp. Nov. the lowest being 0.68 (mg/gm wet wt) in winter season and highest being 0.79 (mg/gm wet wt) in summer season.

*Cotugnia pratibhae* sp. Nov the lowest being 0.42 (mg/gm wet wt) in winter season and highest being 0.65 (mg/gm wet wt) in rainy season.

*Cotugnia govindi* sp. Nov. the lowest being 0.88 (mg/gm wet wt) in summer season and highest being 0.65 (mg/gm wet wt) in rainy season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed) the lowest being 0.76 (mg/gm wet wt) in rainy season and highest being 0.95 (mg/gm wet wt) in summer season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed) the lowest being 0.76 (mg/gm wet wt) in winter season and highest being 0.85 (mg/gm wet wt) in summer season.

*Raillietina (R) fuhrmanni*, Southwell, 1922 (Redescribed) the lowest being 0.65 (mg/gm wet wt) in winter season and highest being 1.03 (mg/gm wet wt) in summer season.
Raillietina (R) quadritesticulata, Moghe, 1925 (Redescribed) the lowest being 0.81 (mg/gm wet wt) in rainy season and highest being 0.98 (mg/gm wet wt) in summer season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). the lowest being 0.79 (mg/gm wet wt) in rainy season and highest being 1.01 (mg/gm wet wt) summer season.

From the table No. 5 and graph G-5 showing the lactate content highest being 1.03 (mg/gm wet wt) Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed) in summer season. and lowest being 0.42 (mg/gm wet wt) Cotugnia pratibhae sp. Nov the in winter season.
LACTATE DEHYDROGENASE

Introduction:

Lactate dehydrogenase is an important enzyme in the Glycolytic metabolism. It is an enzyme which is responsible for the reduction of pyruvate to lactate. Lactate dehydrogenase is linked to pyridine nucleotide co-enzyme except in bacteria & in year specific to substrate L.Lactate. Therefore lactate dehydrogenase is described as L.lactate, Nicotinamide Adenine Dinucleotide (NAO) oxidoreductase. The most extensively studied lactate dehydrogenase is the ones from the skeletal & heart musde of vertebrates. The structure of lactate dehydrogenase (LDH) muscle is studied by using ‘X’ray crystallographic techniques. The molecule of LDH is made up of four sub-units consisting of two types. i.e. ‘M’ & ‘H’. The relative proportion of ‘M’ & ‘H’ tyoe would characterise the five isoenzyme of LDH.

In facultative and obligate quaerobes, the pyruvate is reduced to lactate and in the process, the NADH produced by the glyceraldehydes phosphate dehydrogenase is reoxidised to NAD+ which insures an uninterrupted glucolysis. The lactate which is acidic & not useful to the parasitic helminthes is excreted out (Barrett John 1982).

In anaerobic parasites the lactate produced id oxidised to pyruvate to be incorporated into Kreb’s cycle but in the aerobic metabolism this pyruvate is reduced to lactate thus the reaction is reversible depending on the requirement of the parasites. (Read 1951) Lactate dehydrogenase has been investigated in a number of helminth parasites. Read (1951) reported that the LDH activity in Hymenolepis diminuta, Waitz, (1963)
reported the presence of LDH in both the larval & adult Hydatigera taeniformis. Rothman and Lee (1963) detected the LDH activity in epidermis of Hymenolepis citilli, Moon et al. (1977) on *Hymenolepis diminuta* and (Pappas and Schroder, 1979) Hymenolepis microstomata has made a study on various aspects of the activity of L.D.H.

The number of LDH isoenzyme present in different tissue is found to be variable. Walkey and Fairbairn (1973) demonstrated two iso-enzymes using electrophoretic technique. Moon et al., (1977) demonstrated two and four isoenzyme in purified and unpurified enzyme preparation of *Hymenolepis diminuta* respectively. Logan et al. (1977) also demonstrated the pressures of two iso-enzyme by starch gel electrophoresis method in *Hymenolepis diminuta*. Lactate dehydrogenase showed decreased stability at higher temperature (Pappas and Schroeder, 1979) but has more stability than other dehydrogenase, In *Hymenolepis diminuta* the LDH was inhibited by the substrate concentration.

**Latate dehydrogenase cl-lactate, nad oxidired uctase : ec 1.1.1.27)**

**Method:**

The worm were weighed and homogenized in ice cold 0.25m sucrose solution. The homogenates was centrifuged for 15 min at 2500rpm. The supernatant was used for the enzyme assay by modified of Nachla’s et al., (1960)

**Principle:**

Tetrazolium salts are a unique class of oxidation reduction indicator. They give a stable colored formation on reduction. They are highly soluble in aqueous solution and they are easily permeable through
membranes. The different tetrzolium salts receive electrons from different steps of electron transport system (Nachloos et al., 1960)

**Reagents required:**

1. **0.25m Disodium phosphate solution:** 4.272 gm of disodium phosphate salt was dissolved in 100 ml of double distilled water.

2. **0.25 M monopotssium sulphate solution:** In 100ml of double distilled water 6.528gm of monopotassium salt was dissolved.

3. **Phosphate buffer pH 7.4:** The solution 1 and 2 were mixed and pH was adjusted to 7.4.

4. **0.001 M NAD solution:** In 100 ml of double distilled water, 7 mg of NAD was dissolved.

5. **0.1 sodium lactate solution:** 2.2 ml 50% sodium lactate solution was made up to 100 ml.

6. **0.004 M INT solution:** In 100ml of double distilled water, 200mg of 2-p-Iodophenyl, 3-p-Nitropheneyl, 5-phenyl Tetrazolium chloride was dissolved.

7. **0.25m sucrose solution:** In 100ml of double distilled water, 85.5 gm of sucrose was dissolved.

**Procedure:**

Incubation mixture consists of:

1. 0.4ml sodium phosphate buffer (pH7.4)
2. 0.5 ml of 0.1 M sodium lactate solution (pH 7.4)
3. 0.1 ml INT
4. 0.1ml NAD
5. 0.5 ml homogenate.

The 2 ml of incubation mixture was incubated for 30 minutes at 37 ± 1°C 4ml of glacial acetic acid to stop the reaction. The formazan formed was extracted by shaking with the addition of 4 ml of toluence and left overnight in the refrigeration. The intensity of the colour of the formazan was read at 495 nm using toluence as blank in spectrophotometer.

The lactate dehydrogenate content was calculated from the standard graph and expressed by moles of formazan 1 mg protein/hour.

The data pertaining to the LDH content in the tissue of the host and parasite is shown in table No.6 and graphically in the graph No.6. The LDH content in the cestode infected intestinal tissue of *Gallus gallus domesticus*. 
Mean values of (LDS) content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

Table No. 6

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Cestode parasites</th>
<th>LDH content (µ mole of formazan/mg protein/hour)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>parasites</td>
<td>Winter</td>
<td>Summer</td>
<td>Rainy</td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>2.22 2.32 2.12</td>
<td>12.61</td>
<td>11.76</td>
<td>11.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>2.22 2.44 2.15</td>
<td>12.84</td>
<td>12.25</td>
<td>13.05</td>
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</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>2.25 1.98 2.06</td>
<td>12.75</td>
<td>12.14</td>
<td>11.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>2.28 1.91 2.07</td>
<td>12.57</td>
<td>11.68</td>
<td>12.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed).</td>
<td>2.24 2.15 1.98</td>
<td>12.79</td>
<td>12.11</td>
<td>10.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed).</td>
<td>1.92 2.05 2.13</td>
<td>12.89</td>
<td>12.17</td>
<td>10.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed).</td>
<td>1.90 1.98 2.03</td>
<td>12.58</td>
<td>11.35</td>
<td>13.01</td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Raillietina (R) quadritesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>1.85 2.01 1.91</td>
<td>12.89</td>
<td>13.48</td>
<td>10.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Raillietina (R) friedbergeri</em>, (Linstow, 1978), Fuhrmann, 1920 (Redescribed).</td>
<td>1.96 1.98 2.01</td>
<td>12.60</td>
<td>12.71</td>
<td>11.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Graph shows Mean values of LDH content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

**Graph - 6**

**LDH content (µ mole of formazan/mg protein/hour)**
- Parababylinae Winter
- Parababylinae Summer
- Parababylinae Rainy

**Intestinal tissue**
- Winter
- Summer
- Rainy
Result and Observation:

Data showing LDH content of the worm, reveal that there is little variation in the contents of *Cotugnia* and *Raillietina* species in the different season of the year.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 2.12 (µ mole of formazan/mg protein/hour) in rainy season and highest being 2.32 (µ mole of formazan/mg protein/hour) summer season.

*Cotugnia hyderabadensis* sp. Nov. The lowest being 2.15 (µ mole of formazan/mg protein/hour) in rainy season and highest being 2.44 (µ mole of formazan/mg protein/hour) in summer season.

*Cotugnia pratibhae* sp. Nov. The lowest being 1.98 (µ mole of formazan/mg protein/hour) in summer season and highest being 2.25 (µ mole of formazan/mg protein/hour) in winter season.

*Cotugnia govindi* sp. Nov. the lowest being 1.91 (µ mole of formazan/mg protein/hour) in summer season and highest being 2.28 (µ mole of formazan/mg protein/hour) in winter season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed) the lowest being 0.76 (µ mole of formazan/mg protein/hour) in rainy season and highest being 0.95 (µ mole of formazan/mg protein/hour) in summer season.

*Raillietina (R) singhi*, Malviya, 1971 (Redescribed) the lowest being 1.98 (µ mole of formazan/mg protein/hour) in rainy season and highest being 2.24 (µ mole of formazan/mg protein/hour) in winter season.
Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed) the lowest being 1.92 (µ mole of formazan/mg protein/hour) in winter season and highest being 2.13 (µ mole of formazan/mg protein/hour) in rainy season.

Raillietina (R) quadritesticulata, Moghe, 1925 (Redescribed) the lowest being 1.85 (µ mole of formazan/mg protein/hour) in winter season and highest being 2.01 (µ mole of formazan/mg protein/hour) in summer season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 1.96 (µ mole of formazan/mg protein/hour) in winter season and highest being 2.01 (µ mole of formazan/mg protein/hour) summer season.

From the table No. 6 and graph G-6 showing the lactate content highest being 2.44 (µ mole of formazan/mg protein/hour) Cotugnia hyderabadensis sp. Nov in summer season and lowest being 1.85 (µ mole of formazan/mg protein/hour) Raillietina (R) quadritesticulata, Moghe, 1925 (Redescribed) in winter season.
SUCCINATE DEHYDROGENASE

Introduction:

Succinate dehydrogenase (S.D.H.) is one of the key enzyme in the Kreb’s cycle and this enzyme catalyses the removal of two hydrogen atoms from succinic acid to formic acid. This is the only reaction in Kreb’s cycle in which pyridine nucleotide does not participate. The hydrogen atom is liberated in presence of Succinate dehydrogenase (S.D.H.) enzyme which is accepted by flaoproteins. These atoms are transferred to the electron transport system for complete oxidation.

SDH is the only enzyme of Kreb’s cycle which is bound to the inner membrane of mitochondria. The flavin is co-valantly linked to protein. The important function of SDH is to transfer electrons to the respiratory chain. The enzyme was purified by (Davis and Hatefi, 1971) from beef heart mitochondria. SDH consists of two disimilar sub units. The larger sub-unit carry the co-valantly linked flavin in the presence of suitable electron donor such as FMMH2.

Succinate dehydrogenase has been estimated biochemically in *Hymenolepis diminuta* (Read 1952) H.Nang (Goldberg and Nolf 1954) *Taenia taeniformis* (Pennoit Cooman and Van Grambergan, 1942) Read and Simmons, 1963) and *Moniezia benedini* (Van Grembergen,1947). Agosin and Repetto (1963) determined the enzyme activity biochemically in the scolices of *Echinococcus granulosus*, *Cotugnia dignopora* (Simha et.al.1981) and trematodes *Schistosoma mansoni* (Coles, 1972)
The pressure of SDH was established in Hymenolepos citelli (Rothman 1966) H. diminuta (Heneman and Voqé 1960) and Cysticercoid larva of Hymenolepis nang (Heyneman and Voqé, 1960).

The significant end product of carbohydrate fermentation is succinate (Schiebel et al., 1968) Glycolysis in H. diminuta is largely diverted at the stage of phosphoenol pyruvate towards the synthesis of oxalacetate by CO2 fixation (Bueding and Saz, 1968) and from oxaloacetate, succinate is low possesses anaerobic metabolism and fixes CO2 Von Brand (1973). Similar type of anaerobic pathway (CO2 fixation) was found in cxestode in expanse (Bryant and Bhem, 1976). The tricarboxylic acid cycle does not appear to operative as an energy yielding pathway is H. diminuta (Schiebel and Saz 1966).

The enzyme succinic dehydrogenase is usually connected to the respiratory chain forming succinic oxidase system. Bueding (1962) emphasized that the succinic oxidase can serve as electron acceptor for succinate and as an electron donor for fumarate. He also indicated that succinic oxidase system reduces formate rapidly but oxidase succinic slowly at low oxygen tension. In M expuasa the main pathway of succinic oxidation seems to be due to flavoprotein with the accumulation of Hydrogen peroxide (Cheah and Bryant, 1966; Cheah 1967).

Thus in these helminth parasites the electron acceptor could be fumarate or oxygen (i.e. anaerobic and aerobic metabolism pathway) as the case may be.
Mean values of SDH content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

Table No. - 7

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Cestode parasites</th>
<th>SDH content (µ mole of formazan/mg protein/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>parasites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winter</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>21.48</td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>22.47</td>
</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>21.38</td>
</tr>
<tr>
<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>20.39</td>
</tr>
<tr>
<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed)</td>
<td>23.32</td>
</tr>
<tr>
<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed)</td>
<td>19.34</td>
</tr>
<tr>
<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed)</td>
<td>15.42</td>
</tr>
<tr>
<td>8</td>
<td><em>Raillietina (R) quadritesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>19.65</td>
</tr>
</tbody>
</table>
Graph shows Mean values of SDH content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*.

**Graph - 7**

- SDH content (µ mole of formazan/mg protein/hour) parasites Winter
- SDH content (µ mole of formazan/mg protein/hour) parasites Summer
- SDH content (µ mole of formazan/mg protein/hour) parasites Rainy

**Intestinal tissue**

- SDH content (µ mole of formazan/mg protein/hour) Intestinal tissue Winter
- SDH content (µ mole of formazan/mg protein/hour) Intestinal tissue Summer
- SDH content (µ mole of formazan/mg protein/hour) Intestinal tissue Rainy
Result and observation:

The quantitative values of SDH content (µ mole of formazan/mg protein/hour) in *Cotugnia shrirampurensis*, *Cotugnia hyderadenesis*, *Cotugnia pratibhæ*, *Cotugnia govindi*, *Cotugnia polyacantha*, *Raillietina (R) singhi*, *Raillietina (R) fuhrmanni*, *Raillietina (R) quadritesticulata*, and *Raillietina (R) friedbergeri* of cestode parasites are shown in table No-7 and graphically represented in graph G-7. The total SDH content data showing SDH content of the worm, reveal that there is little variation in content.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 21.48 (µ mole of formazan/mg protein/hour) in the winter season and highest being 22.72 (µ mole of formazan/mg protein/hour) in the rainy season.

*Cotugnia hyderabadenisis* sp. Nov. The lowest being 22.47 (µ mole of formazan/mg protein/hour) in the winter season and highest being 23.75 (µ mole of formazan/mg protein/hour) in summer season.

*Cotugnia pratibhæ* sp. Nov. The lowest being 21.38 (µ mole of formazan/mg protein/hour) in winter season and highest being 22.51 (µ mole of formazan/mg protein/hour) in summer season.

*Cotugnia govindi* sp. Nov. The lowest being 19.26 (µ mole of formazan/mg protein/hour) in summer season and highest being 20.39 (µ mole of formazan/mg protein/hour) in winter season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed). The lowest being 21.27 (µ mole of formazan/mg protein/hour) in rainy season and highest being 23.32 (µ mole of formazan/mg protein/hour) in winter season.
Raillietina (R) singhi., Malviya, 1971 (Redescribed). The lowest being 18.41 (µ mole of formazan/mg protein/hour) in summer season and highest being 19.37 (µ mole of formazan/ mg protein/ hour) in winter season.

Raillietina (R) fuhrmanni, Southwell, 1922 (Redescribed). The lowest being 15.42 (µ mole of formazan/mg protein/hour) in winter season and highest being 16.36 (µ mole of formazan/mg protein/hour) in summer season.

Raillietina (R) quadristesticulata, Moghe, 1925 (Redescribed). The lowest being 17.75 (µ mole of formazan/mg protein/hour) in rainy season and highest being 19.65 (µ mole of formazan/mg protein/hour) in winter season.

Raillietina (R) friedbergeri, (Linstow, 1978), Fuhrmann, 1920 (Redescribed). The lowest being 4.19 (µ mole of formazan/mg protein/hour) in the rainy season and highest being 6.12 (µ mole of formazan/mg protein/hour) summer season.

From the above observation, the SDH content shows variability in different species of Cotugnia and Raillietina genera. The SDH content is highest in Cotugnia hyderabadensis sp. Nov. (23.75 µ mole of formazan/ mg protein/ hour) in summer season. The cholesterol content is lowest in Raillietina (R) fuhrmanni, Southwell, 1922 (15.42 µ mole of formazan/mg protein/ hour) in winter season.
CHOLESTEROL

Introduction:

Cholesterol is a steroid and occurs in the unsaponifiable residue of lipid. Cholesterol is widely distributed in all the tissue of animal. The parasite has a high content of cholesterol and of neutral and phospholipids which content large amount polyunsaturated fatty acids. The fatty acid composition of the parasite closely resembles that of its host.

Cholesterol was identified in the few cestodes, in *Hymenolepis diminuta* cyst of *Echinococcus granulosus* (Smyth, 1969) in *Diphyllobothrium latum* (Faust and Tallquist.,1907) in *Cysticercus fasciolaris* (Salisbury and Anderson, 1939) Von Brand (1933) isolated a sterol from the unsaponifiable fractions of *Moniezia expansa* (Cmelik, 1952) studied the unsaponifiable matter from cyst membrane of *Echinococcus granulosus* and found that 75% of this unsaponifiable matter was cholesterol. Thompson et al., (1960), studied the cholesterol content in two cestodes *Hydatigera taeniformis* and *Moniezia expansa* reported that 98% and 85% of unsaponifiable matter contains is cholesterol.

It is not surprising that the cholesterol was most abundant sterol in all the tape worms examined since it is the most abundant sterol in the body fluids of at least one of the host species. Cholesterol is the only sterol present in lipids of *H. diminuta* (Ginger and fairbairn, 1966 b) and although not synthesized do Novo, it is readily absorbed by this species and by others (frayha and fairbairn, 1968, 1969) Meyor et al., 1966,
frayha 1968). Cholesterol was accompanied by two minor sterols which are most abundant in the worm from the rectal gland (George H.Buteau, 1976) cestodes are not capable of the synthesizing cholesterol, although they can esterify it. Incubation with common cholesterol precursor such as acetate and malvionate gave uniformly negative result in E.granulosus (Frayha, 1968), H.diminuta (Frayha and fairbairn, 1969) and Moniliformis clabis (Barrett etal 1970) HDL & its major component; apolipoprotein A-1 (apoA-1) play a central role in reverse cholesterol transport. The microstomal fractions of the cells are responsible for the biosynthesis of the cholesterol. Acetyl coenzyme A is the source of all carbon atoms in cholesterol.

For the present study the fully mature worms of the same size are used in assaying cholestero content in the different regions of the worm.

**Cholesterol Method :**

The whole worms were homogenized in 3:1 Alcohol-Ether mixture in ice cold to approximately 4°c. The cholesterol content was estimated by the method described by Crawford (1958)

**Principle :**

The homogenate prepared in alcohol other mixture reacts with sulphuric acid in the presence of ferric chloride to form a stable brown colour.

**Reagents required :**

1. **10% ferric chloride** : In 100ml of Glacial acetic acid 10 gm of ferric chloride was dissolved.
2. **3:1 Alcohol Ether mixture**: The mixture was prepared by the addition of 300 ml of absolute alcohol and 100 ml of ethyl.

3. **Colour reagent**: 0.5 ml of 10% FeCl₃ was added to 7.5 ml conc. H₂SO₄. This solution was mixed well and diluted to 50 ml with conc. H₂SO₄. The reagent initially appear clear and light yellow in colour but becomes the colourless gradually. This colour reagent was prepared freshly before use.

4. **Cholesterol standard solution**: The stock solution was prepared by dissolving 100 mg of pure dry cholesterol in 100 ml of glacial acetic acid. Then 20 ml of this stock standard was diluted to 100 ml with glacial acetic acid. These standard solutions contain 0.2 mg cholesterol.

**Procedure**

**Test sample**:

To 1 ml of homogenate extract 6 ml of glacial acetic acid was added in a boiling tube. 4 ml of colour reagent was added slowly down the sides of boiling tube which form a layer below the acetic distribution. The tubes were allowed to cool. Blank consisting of 1 ml of the water instead of homogenate extract.

**Standard**:

To 1 ml of cholesterol standard solution 5 ml of glacial acetic acid, 0.1 ml of water added, 4 ml of colour reagent was added and treated as a test sample. The intensity of colour was read at 540 nm against the blank in the spectrophotometer.
Mean values of Cholesterol content in following cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

Table No. 8

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Cestode parasites</th>
<th>Cholesterol content (mg/gm fresh wt)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Winter</td>
</tr>
<tr>
<td>1</td>
<td><em>Cotugnia shrirampurensis</em> sp. Nov.</td>
<td>7.18</td>
</tr>
<tr>
<td>2</td>
<td><em>Cotugnia hyderabadensis</em> sp. Nov.</td>
<td>7.35</td>
</tr>
<tr>
<td>3</td>
<td><em>Cotugnia pratibhae</em> sp. Nov.</td>
<td>8.75</td>
</tr>
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<td>4</td>
<td><em>Cotugnia govindi</em> sp. Nov.</td>
<td>8.21</td>
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<td>5</td>
<td><em>Cotugnia polyacantha</em>, Fuhrmann, 1909 (Redescribed)</td>
<td>7.12</td>
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<td>6</td>
<td><em>Raillietina (R) singhi</em>, Malviya, 1971 (Redescribed)</td>
<td>6.14</td>
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<td>7</td>
<td><em>Raillietina (R) fuhrmanni</em>, Southwell, 1922 (Redescribed)</td>
<td>5.52</td>
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<td>8</td>
<td><em>Raillietina (R) quadritesticulata</em>, Moghe, 1925 (Redescribed)</td>
<td>6.31</td>
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</tbody>
</table>
Graph shows Mean values of Cholesterol content in cestode parasites and infected intestinal tissue in the *Gallus gallus domesticus*

**Graph - 8**

- Cholesterol content (mg/gm fresh wt) parasites Winter
- Cholesterol content (mg/gm fresh wt) parasites Summer
- Cholesterol content (mg/gm fresh wt) parasites Rainy

Cholesterol content in cestode parasites and infected intestinal tissue.
Result and observation:

The quantitative values of cholesterol content in *Cotugnia shrirampurensis*, *Cotugnia hyderadensis*, *Cotugnia pratibhae*, *Cotugnia govindi*, *Cotugnia polyacantha*, *Raillietina (R) singhi*, *Raillietina (R) fuhrmanni*, *Raillietina (R) quadritesticulata*, and *Raillietina (R) friedbergeri* of cestode parasites are shown in table No-8 and graphically represented in graph G-8. The total cholesterol content, data showing cholesterol content of the worm, reveal that there is little variation in content.

*Cotugnia shrirampurensis* sp. Nov. The lowest being 6.14 (mg/gm fresh wt) in the rainy season and highest being 7.98 (mg/gm fresh wt) summer season.

*Cotugnia hyderabensis* sp. Nov. The lowest being 6.29 (mg/gm fresh wt) in the rainy season and highest being 7.68 (mg/gm fresh wt) summer season.

*Cotugnia pratibhae* sp. Nov. The lowest being 7.18 (mg/gm fresh wt) in the rainy season and highest being 9.15 (mg/gm fresh wt) summer season.

*Cotugnia govindi* sp. Nov. The lowest being 6.14 (mg/gm fresh wt) in the rainy season and highest being 9.05 (mg/gm fresh wt) in the summer season.

*Cotugnia polyacantha*, Fuhrmann, 1909 (Redescribed) the lowest being 5.48 (mg/gm fresh wt) in the rainy season and highest being 8.01 (mg/gm fresh wt) summer season.
**Raillietina (R) singhi**, Malviya, 1971 (Redescribed) the lowest being 5.05 (mg/gm fresh wt) in the rainy season and highest being 7.15 (mg/gm fresh wt) summer season.

**Raillietina (R) fuhrmanni**, Southwell, 1922 (Redescribed) the lowest being 4.14 (mg/gm fresh wt) in the rainy season and highest being 5.92 (mg/gm fresh wt) summer season.

**Raillietina (R) quadritesticulata**, Moghe, 1925 (Redescribed) the lowest being 4.78 (mg/gm fresh wt) in the rainy season and highest being 6.81 (mg/gm fresh wt) summer season.

**Raillietina (R) friedbergeri**, (Linstow, 1978), Fuhrmann, 1920 (Redescribed) the lowest being 4.19 (mg/gm fresh wt) in the rainy season and highest being 6.12 (mg/gm fresh wt) summer season.

From the above observation, the cholesterol content shows variability in different species of *Cotugnia* and *Raillietina* genera. The cholesterol content is highest in *Cotugnia pratibhae* sp. Nov (9.15mg/gm fresh wt) in summer season. The cholesterol content is lowest in *Cotugnia polyacantha*, Fuhrmann, 1909 (4.14mg/gm fresh wt) in rainy season.