

Some quantitative studies of carbohydrate metabolites in cestode parasite of *Gallus gallus domesticus*

Waghmare S.B.* and Chavan R.J.

*Helminth Research Lab, Department of Zoology, Dr. B.A.M. University, Aurangabad, MS, sachinash_red@yahoo.co.in

Abstract- Present investigation includes the quantitative estimation of carbohydrate metabolism i.e., total glycogen, pyruvate, lactic acid, lactate dehydrogenase, malate dehydrogenase, phosphatases activity in cestode species of *Gallus gallus domesticus*. The carbohydrate metabolism activity were observed in the intestinal parasites *Cotugnia digonopora* (Pasquale, 1890) were capable of extracting nutrient material from their host and thus represented high level in carbohydrate metabolism. The significance of various amount of pyruvate in anaerobic intestinal parasites and various factors of its role was also discussed.

Key words: Carbohydrate metabolism/Pyruvate/ SDH/MDH/ Cestode parasites/*Gallus gallus domesticus*

Introduction

Glycogen is the starting material in carbohydrate metabolism. It may be first degraded to glucose. The subsequent steps involved, referred to as glycolysis or EMP pathway occur in the cytoplasm. Thus can be divided in to two phases the preparatory and oxidative phases. In certain helminth parasites polysaccharides content varies with age in *Hymenolepis* sp and *Cystercus fasciolaris* an increased trend of glycogen content was observed as age increases (Lewert and Lec, 1955) variability in glycogen content in different helminth parasites is also dependent on the oxygen content in inhibition. Parasites is also dependent on the oxygen content in inhabitation. Parasite living in O₂ deficient environment has high content of glycogen in on the contrary, glycogen levels are less in O₂ rich environment, Goll, 1957. In cestodes appreciable quantity of glycogen is present in medullary parenchyma and also in uterine egg's. The parasites living in anaerobic or semi-anaerobic habitat's gain their energy almost exclusively through the fermentation of carbohydrate because it is a much better substratum for gaining anaerobic energy than either protein or fat. The most common reserve carbohydrate is glycogen. among helminth, highest rate has been observed in *schistosomes* with an hourly rate of about 20 % of their dry weight (Bueding peter's and Waite, 1947).

Material and Methods

The flatworm *Cotugnia digonopora* (Pasquale, 1890), were collected and homogenized in potter – Elvehjem tissue grinder to a concentration of 10 % (w/v) in modified mitochondrial medium (Scheibel et al, 1968) of the composition 0.32 M sucrose, 0.15 % crystalline bovine serum albumin and 0.005 M EDTA, P^H 7.4. The homogenate was centrifuged at 800 rpm for 20 min followed by recentrifugation of supernate at 15000 rpm for 30 min. The mitochondrial pellet was centrifuged twice by suspending it in the modified mitochondrial medium and centrifuged at 15000 rpm. The washed pellet was faunally suspended in 0.05 M Tris HCL buffer P^H 7.4 and designated as 'mitochondria'. The superstate, obtained above after the removal of

mitochondrial pellet was further centrifuged at 105,000 rpm for 1 hour to obtain the cytosol fraction. Mitochondrial fraction was disrupted by 4 successive bursts for 39 s each (with 1 min cooling intervals) using a Heat cell sonicator, model W.220-F. All the above operation was carried out at 4^o C for quantitative assay.

For Enzyme assay: - Spectrophotometric assay as described elsewhere (Mc Manus and Smyth, 1982) were carried out for Lactate dehydrogenase (LDH) [EC 1.1.1.27], Malate dehydrogenase (MDH) [EC 1.1.1.37; reduction of oxaloacetate to malate] and phosphatases (Alkaline and Acidic) [EC 3.1.3.1], Succinate dehydrogenase [SDH] (EC 1.3.99.1). Reaction was stopped after 25 min of incubation and pyruvate was estimated in the deproteinized aliquot according to the method of Bueding and Saz (1968).

Colorimetric assay was carried out for the total glycogen estimation by Kemp and Heijhnger (1954) and lactic acid by Baker's and summerson (1941).

Result and Discussion

The quantitative values of some carbohydrate metabolism activities in *Cotugnia digonopora* (Pasquale, 1890), species of cestode parasite are shown in table no 1 and graphically represented in graph no 1. The total glycogen content was 4.521±0.172 mg of glucose/gm of fresh weight of parasite, pyruvate 25.48±4.721 µg of pyruvate/gm of fresh weight of parasite. The lactic acid was 1.053±0.122 mg lactate/gm of fresh weight of parasite. The quantitative study of some enzyme assay of cestode parasite was Lactate dehydrogenase [E.C. 1.1.1.27] 3.532±0.117 µ moles of formazan/mg protein /hour, malate dehydrogenase [E.C.1.1.1.37] 7.812±1.127 µ moles of formazan/mg protein /hour and phosphatases, the alkaline phosphatase [E.C. 3.1 3.1] was at P^H 9 0.004±0.0004 and acid phosphatase at P^H 6 0.0017±0.0002 respectively and Succinate dehydrogenase (SDH) [EC1.3.99.1] 10.672±0.754 µ moles of formazan/mg protein/hour.

Glycogen:- The main carbohydrate reserve in parasitic helminth is glycogen which is typical energy reserve of helminth. The early work of Claude Bernard (1859) and Foster (1856) demonstrated the occurrence of glycogen in helminthes. The glycogen content of parasites fluctuates over a wide range, due to factors such as season's, physiological state of the host, the time of autopsy, strain of the host, rate of the infection and stage in life cycle. The glycogen content of few parasites is in percentage of their fresh weight. In *E.granulosus* larva (2.8%) Agosin et al 1957, *Hymenolepis diminuta* from 1.1 to 9.3 % (Fairbairn et al 1961) *Moniezia expansa* from 2.7 to 5.2 % Von brand, 1933). *Cotugnia digonopora* (Pasquale, 1890) is intestinal parasite, where environmental O₂ is not available. These parasites depend on anaerobic carbohydrate metabolism to obtain the energy required, a regular supply of glycogen is necessary. Hence large quantities of polysaccharide are stored which can be oxidized to yield ATP.

Pyruvate:- The small amount of phosphoenol pyruvate is converted into pyruvate by enzyme pyruvate kinase and then reduced to lactate by LDH and NADH. Mg⁺ and K ion required as activators. The cestode parasites obtain part of energy through the glycolysis anaerobic pathway or EMP pathway, the end product is pyruvate from the pyruvate formation of amino acid is done by transamination. Pyruvate content is also correlated to the glycogen content of parasites, pyruvate is having important role in the energy metabolism of cestode. The pyruvate latter gets converted by action of LDH into lactate which is excreted out along with energy rich product which is utilized by host.

Lactic acid:- The major end products are lactic acid and succinic acid, which product gets accumulated can be usually linked to the activities of pyruvate kinase (PK) phosphoenol pyruvate carboxykinase (PEPCK) and of LDH and MDH (Bryant 1975) Moon et al (1977) have shown that regulatory properties of PK and PEPCK are responsible for the accumulation of lactic acid and succinic acid respectively. The production of lactate and its excretion without being further metabolized is certainly more acidic and less energy yielding than the production of succinate (Hochoka and Somero, 1973).

Lactate dehydrogenase:- There is the presence of LDH in *Cotugnia digonopora* (Pasquale, 1890) parasite. The enzymes show activity in *Cotugnia digonopora* (Pasquale, 1890), when the result correlated to that of lactic acid content in the same species, they reveal that higher the lactic acid content, the LDH activity is also high, LDH brings about reduction of pyruvate and results in production of lactic acid and thereby supplying an ATP molecule. LDH is helpful not only from the

energy point of view but also in the maintenance of the cytoplasmic redox state.

Malate dehydrogenase:- MDH is linked to co-enzymes NADH which is responsible for the conversion of malate to oxaloacetate in the TCA cycle. This enzyme is found in large amounts in mitochondria and sarcosomes and also in cytoplasm, MDH was demonstrated in *hymenolepis diminuta* (Read 1952, 1953, Waitz and Schardein 1964 Bueding and Saz 1961, *Moniezia benedini* (Pennoit De Coomen and Van Grembergen 1942), *Fasciola hepatica* (Pennoit De Coomen and van Grembergen 1942). The LDH in parasitic helminth is known to produce the malate from oxaloacetate (Bueding and Saz 1968) and linked to NADH co-enzymes, whereas another type of MDH which converts the malate to pyruvate by dismutation is also reported by Saz and Hubbard 1957 and Saz and Lescure 1969. Reeves (1970) has shown that production of pyruvate from malate is linked to NADH co-enzymes. The activity of MDH is very high in the direction of malate formation than activity of LDH.

Succinate dehydrogenase:- One of the key enzymes in Krebs's cycle this enzyme catalyses the removal of two hydrogen atoms from succinic acid to form fumaric acid. This is only reaction in Krebs's cycle in which pyridine nucleotide does not participate. SDH is to transfer electron to respiratory chain. SDH was estimated biochemically in *H.diminuta* (Read, 1952), *H.nana* (Goldberg and Nolf, 1954), *Taenia pisciformis* (Pennoit De Cooman and Van Grembergen, 1942). Result revealed that SDH activity is very high in the avian parasite. This gives evidence that Krebs's cycle is operating in these parasites. High level of SDH activity found in *Cotugnia digonopora* (Pasquale, 1890) suggests the existence of CO₂ fixation pathway or partial reverse of Krebs's cycle.

Phosphatases:- In the present investigation only the phosphomonoesterases groups are studied they were differentiated into acid and alkaline phosphatases. Bogtish (1960) showed iso-enzymes of the alkaline phosphatases in the *posthodiplostomum minimum* and Meyer's (1966) observed five isoenzymes with acid phosphatase activity in *Ditylenchus trifurcatus*. Although the phosphatases have been found rather frequently associated with structure of absorption like tegument of cestodes, intestinal cell of the nematodes and excretory system of trematodes (Coil 1958, Cheng 1954). It is evident that both acid and alkaline phosphatases are present in *Cotugnia digonopora* (Pasquale, 1890) alkaline phosphatase is predominant over acid phosphatase. These results agree with similar results reported by Leafverve (1952) in *Taenia taeniformis*, Erasmus (1957 a, b) in *Taenia pisiformis* and *Moniezia expansa* Waitz and Schardein (1964) and Reddy in *Cotugnia*

digonopora (Pasquale, 1890) and *Railletina tetragona*. In contrast to predominance of alkaline phosphatases in adult cestode, the larval forms have a predominance of acid phosphates (Erasmus.1957 .Arme 1966).The reason for this change in relative proportion of these enzyme in different phases of life history is not clear but it may be associated with the growth and development of the reproductive system in adult worms as observed by Arme (1966).

Acknowledgement

Author Mr. S.B. Waghmare is thankful to University Grants Commission for providing financial assistance through UGC-RFSMS scheme during research work.

References

- [1] Alison W.S. (1966) *Methods Enzymol.*, 9,210.
- [2] Bernard C. (1859) *Quoted by Von Brand (1973). In "Biochemistry of Parasites", Acad .Press, Newyork*
- [3] Bueding.G.(1949) *J.Gen.Physiol.*33.475-495.
- [4] Bodansky D. (1961) *In standard methods of clinical chemistry (ed.D.Seligson) (New York: Academic Press) 3, 182.*
- [5] Erasmus D.A.(1957) *Parasitol.*47,81-91
- [6] McManus D.P. and Smyth J.D. (1982) *Parasitology*, 84.351.
- [7] Mal L. (1964) *J. Parasitol* 50:235-240
- [8] Pampori N.A., et.al.,(1984) *J.Helminthol*,58,39.
- [9] Saz H.J.(1981) *Annu.Rev.Physiol.*,43, 323.
- [10] Read C.P. Jr. (1953) *Exp .Parasitol .2:341-347.*
- [11] Read C.P. (1959) *The role of carbohydrate in the biology of cestodes VIII .Some conclusion and hypothesis 8:365-382.*
- [12] Rhodes M.B., Marsh C.L. and Kelly G.W. (1964) *Expt. Parasit.*,15 (5):403-409.
- [13] Shinde G.B. et.al. (1985) *Di. Parasit.* 2(46):141-152.
- [14] Von Brand T. (1979) *Biochemistry and physiology of endoparasites (Oxford, Amsterdam, New York: Elsevier/NH and Biomed .Press).*
- [15] Walky M. and Fairbairn D. (1973) *J.Exp.Zool.*183. 365-374.

Table 1- Some carbohydrate metabolites in *Cotugnia digonopora* (Pasquale, 1890)

Sr No	Biochemical content.	mg/gm of fresh weight of Parasite.
1	Total Glycogen	4.512±0.172
2	Pyruvate	20.482±4.721
3	Lactic acid	1.553±0.122

Graph 1-Some carbohydrate metabolite in *Cotugnia digonopora* (Pasquale, 1890)

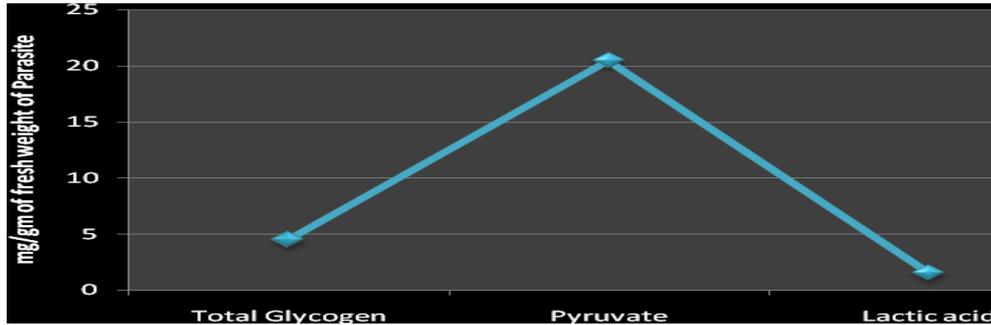


Table 2- Some enzyme carbohydrate metabolites in *Cotugnia digonopora*(Pasquale, 1890)

Sr No	Enzyme content In <i>Cotugnia digonopora</i>	μ moles of formazon/mg protein /hour
1	LDH	3.532±0.137
2	MDH	7.812±1.127
3	SDH	6.587±0.754
4	Acid phosphotases	0.655±0.0001
5	Alkaline phosphotases	0.254±0.0002

Graph 2- Some Enzymes of carbohydrate metabolite in *Cotugnia digonopora* (Pasquale,1890)

