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Histochemical Localisation of Lactate Dehydrogenase in Schistosoma Spindale

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Abstract: Histochemical localization and distribution of Lactate Dehydrogenase has been demonstrated in the digenetic trematode, Schistosoma spindale. It is a fluke inhabiting the portal and mesenteric veins of Bubalus bubalis. Lactate dehydrogenase is the only simple dehydrogenase where both structure and sequence are known. It is responsible for reduction of pyruvate to lactate or in the oxidation of lactate to pyruvate in the last reversible steps of glycolysis. The reaction for LDH enzymes in the various regions of the S. spindale, the tegument, body wall, oral sucker, and ventral sucker were observed. The presence of LDH activity indicates fairly high concentration of lactate which is suggestive of the fact that more carbohydrates are channelled towards lactate production. These results suggest an important role played by lactate dehydrogenase enzyme in the metabolism of the blood fluke S. spindale.
Keywords: Schistosoma spindale, Lactate Dehydrogenase(LDH), Bubalus bubalis, Trematode.

I. INTRODUCTION

Enzymes are vital components of biological systems; as the catalysts of most biochemical reactions they are essential for the metabolic processes occurring within the tissue. Microscopic enzyme techniques are of potential value in detecting histological and cytological alterations which are not discernible with routine non-specific stains (1). Definitive microscopic histochemical techniques utilize tissue sections, the architecture of which is not grossly disturbed, and employ known chemical reactions to produce insoluble chromogenic precipitates precisely at the cytological sites associated with the specific enzyme activities. Oxidation – reduction reactions which take place in biological systems, are characterized by hydrogen atoms or electrons being transferred from molecule to molecule.

Dehydrogenases are the first compounds of the chain of enzymes, which transfer the hydrogen atoms from the substrate to the molecular oxygen (2). Glucose is the major source of energy in animals both during normal and altered physiological conditions. Glycolysis is the oxidation of glucose or glycogen to pyruvic acid or lactic acid, the reactions of glycolysis are similar both in anaerobic and aerobic conditions except for the resultant end products. Pyruvate is the end product of aerobic glycolysis and lactate in the anaerobic conditions.

However, lactate is reconverted into pyruvate when the anaerobic phase is followed by an aerobic phase. The reoxidation of NADH to NAD^+ occurs by the production of lactate by lactate dehydrogenase thus ensuring an uninterrupted glycolysis even in the absence of oxygen by generation of sufficient NAD^+. Lactic acid is the most important end product of both aerobic and anaerobic glycogen fermentation. (3).

The formation in the body of pyruvic acid from lactic acid and the demonstration by Embden and Oppenheimer that pyruvic acid gives aceto acetic acid as well as alanine in the liver, places lactic acid in quite an exceptional position, having a direct metabolic relations with characteristic products of fat, carbohydrate and protein metabolism. The mutual interconversion of lactic acid, glucose and glycogen seems to play a part in the metabolism of most tissues (4). LDH is the only simpler dehydrogenase where both structure and sequence are known. This enzyme is found mainly outside the intracellular organelles, in soluble phase of the cell. Moreover, as explained earlier, lactic acid or lactate from pyruvate assumes a key role in the fat, carbohydrate and protein metabolism (4). Thus the enzyme lactate dehydrogenase involved in the interconversion of lactate and pyruvate has a very important role to play in the metabolism of most tissues(5). Lactate dehydrogenase mediated responses are reversible reactions. Bueding and Most(1953) pointed out that the Embden-Meyerhof scheme of glycolysis had been observed in every helminth and reported that lactic acid production varied in different parasites[6]**.** Coles (1975) reported that schistosomes have a high level of lactate dehydrogenase and pyruvate kinase[7]. The expression and characterization of Lactate dehydrogenase from *Schistosoma japonicum* was done by Lu G et al., 2006[8]. The present study details the localisation of LDH activity in the various sections of *S. spindale* histochemically.

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II. MATERIALS AND METHODS

A. Histoenzymological Studies (Lactate Dehydrogenase)

1) *Method:* Histoenzymological studies involves the localization of enzymes in parasite by using a cold neutral fixative and sectioning the tissue blocks on a cryostat or freeze microtome. 10% neutral buffered formalin was used to fix the cryostat sections 12-16 µ thick. These were put in incubating medium for 30 minutes to 1 hour at 37^{o}C. The sections were immersed in 10% formal saline for 10-15 minutes. Sections were then washed in tap water for 2 minutes. Distilled water was used to rinse the sections and mounted in glycerine jelly. Blue formazon deposits(NBT) were formed at the sites of enzyme activity[9]. The colouration is directly proportional to the amount of the enzyme present in the tissue.

III. RESULTS

The Lactate Dehydrogenase activity localized in the cryostat sections of the parasite were seen as conspicuous blue diformazon granules in the different regions of the parasite. The intensity of the colour in the various tissues of the parasite determined the Lactate dehydrogenase activity. The distribution of LDH activity varied from moderate to intense in the different regions of S. spindale and are as follows

A. The distribution of the enzymatic activity was more pronounced in the tegument and musculature (Fig. 1,2,3,4,5,6,7,13)
B. The muscular layer showed intense activity. The longitudinal muscular layer and circular muscular layer showed fairly elevated LDH activity(Fig 5,6,7,8,9,13).
C. The male and the female parasite showed an considerable activity of LDH (Fig. 1,2,3,4)
D. The muscular layer of suckers which are actively involved in muscular contraction showed localization of lactate dehydrogenase activity (Fig.10,11). A thick bluish colouration of diformazon deposits was observed in the oral sucker and ventral sucker(Fig.12,10,11)

IV. DISCUSSION

The histochemical localization of lactate dehydrogenase in the parasite indicated that more of glycogen is channelized towards glycolysis indicating the catabolic activity to cope up with high energy demands of the parasite. LDH reduces pyruvate to lactate or oxidizes lactate to pyruvate. The process of glycolysis may be accounted to take place at a higher pace. Ryley(1956) had found the pathogenic African trypanosomes excrete at most traces of lactate (10). The malarial parasites produces large amounts of lactic acid accounting in Plasmodium gallinaceum for nearly 100% of the excreted acids (Silverman et al., 1944)[11]. Similar difference exists in helminths. Thus, lactate accounts for practically all the carbon of the glucose metabolized by Dinofilaria uniformis (Von Brand et al., 1963) and for nearly all the carbon in Litomowsoides carinii(Bueding,1949a), Schistosoma mansoni(Bueding,1950; Magzoub and Maegraith,1969; Coles,1972a)[12-16]. Studies on the comparative enzyme histochemistry of immature and mature stages of *Fasciola hepatica* was carried out by Thorpe[17]. Adult *F. hepatica* showed the presence of Lactate dehydrogenase activity. Histochemical demonstration of Lactate dehydrogenase activity was observed in the cuticle of cestodes by Rothman and Lee(1963)[18]. Histochemical localization of lactate dehydrogenase was carried out in Paramphistomum cervi (Trematoda: Paramphistomidae by Patil and Rodgi(1976)[19]. Studies carried out by Mrunalini(1988) have shown weak to intense LDH activity in the tissues of Moniliformis dubius[20]. Bueding(1950) had worked on schistosomes and he proposed that it is a 'homolactic fermemter' **a**s it exclusively converts glucose into lactic acid by glycolysis, a process restricted in the cytosol[14] Energy generation in this process is independent of oxygen and the end product, lactic acid is secreted (Bryant and Behm,1989)[21]. It indicates that the parasites are dependent on this pathway and other energy generating pathways operate. Bueding(1950) found that in vitro glucose utilisation by adult S. mansoni was similar under both an anaerobic and aerobic metabolism and no Pasteur effect was indicated[14]. Bueding and his colleagues(Schiller et al. 1975, Bueding and Fischer, 1982) confirmed that schistosomes are homolactic fermenters and obtain no energy from oxidative pathways[22,23]. Histochemical localization have indicated lactate dehydrogenase activity to be more pronounced in the tegument and the muscular tissue of the parasite. The male and the female showed considerable LDH activity. The regions of the gynaecophoric canal also showed LDH activity. Besides the oral and the ventral sucker showed an intense LDH activity. It can be accounted by the fact that as the blood fluke are constantly involved in contraction, significant amounts of LDH activity have been localized in the sections. In other tissues, its activity varied from moderate to intense. In general, these observations revealed that lactate dehydrogenase is moderately active in some of the tissues and intensely active in few others. This study helps in understanding the energy requirements of the tissues and its vital role in the carbohydrate metabolism. The results of the current investigation are in general agreement with the studies made by Carter and Fairbairn(1975), Coles(1972) in other schistosomes[24,16]

V. CONCLUSION

The histochemical localization of prominent LDH enzyme activity in the sections of the S.spindale suggests that the enzyme reoxidises NADH to NAD through pyruvate lactate pathway and reoxidation of reduced NADH formed during oxidation which is essential for the continuous functioning of the glycolytic pathway. Lactate dehydorgenase is helpful from the energy point of view and also in the maintenance of the cytoplasmic redox state. The restoration of cytoplasmic redox state will help in uninterrupted operation of glycolytic pathway and thus the parasitic helminths are assured for their energy requirements. In S. magrebowiei, Earle(1984) showed that although the male worms converted carbohydrates almost quantitatively to lactate, with glucose-lactate ratio of 1:2, the female and paired worms converted a substantial amount of the glucose via non-glycolytic pathways which may have included oxidative phosphorylation[25]. In parasitic helminths, the lactic acid is excreted out as it is not useful to the parasite and is acidic in nature. The lactate produced under some stress such as muscular contraction is however oxidised to pyruvate which is incorporated into the Kreb's cycle(26,27). It is found that in most of the helminth parasites pyruvate reduction into lactate occurs in preference to lactate oxidation to pyruvate. Studies by Stephanie et al.(2015), have shown that Lactate as a novel quantitative measure of viability in Schistosoma mansoni drug sensitivity assays. Lactate is a sensitive and simple surrogate marker to be measured to determine Schistosoma viability in compound screening assays[28]. Hong You et al(2014) have worked on the revisiting glucose uptake and metabolism in schistosomes and new molecular insights for improved schistosomiasis therapies[29]. The challenges to explore the molecular mechanism required for schistosomes to take up glucose and the role of lactate dehydrogenase in the metabolism to fuel growth can storm new avenues for the novel avenues for the control of schistosomiasis.

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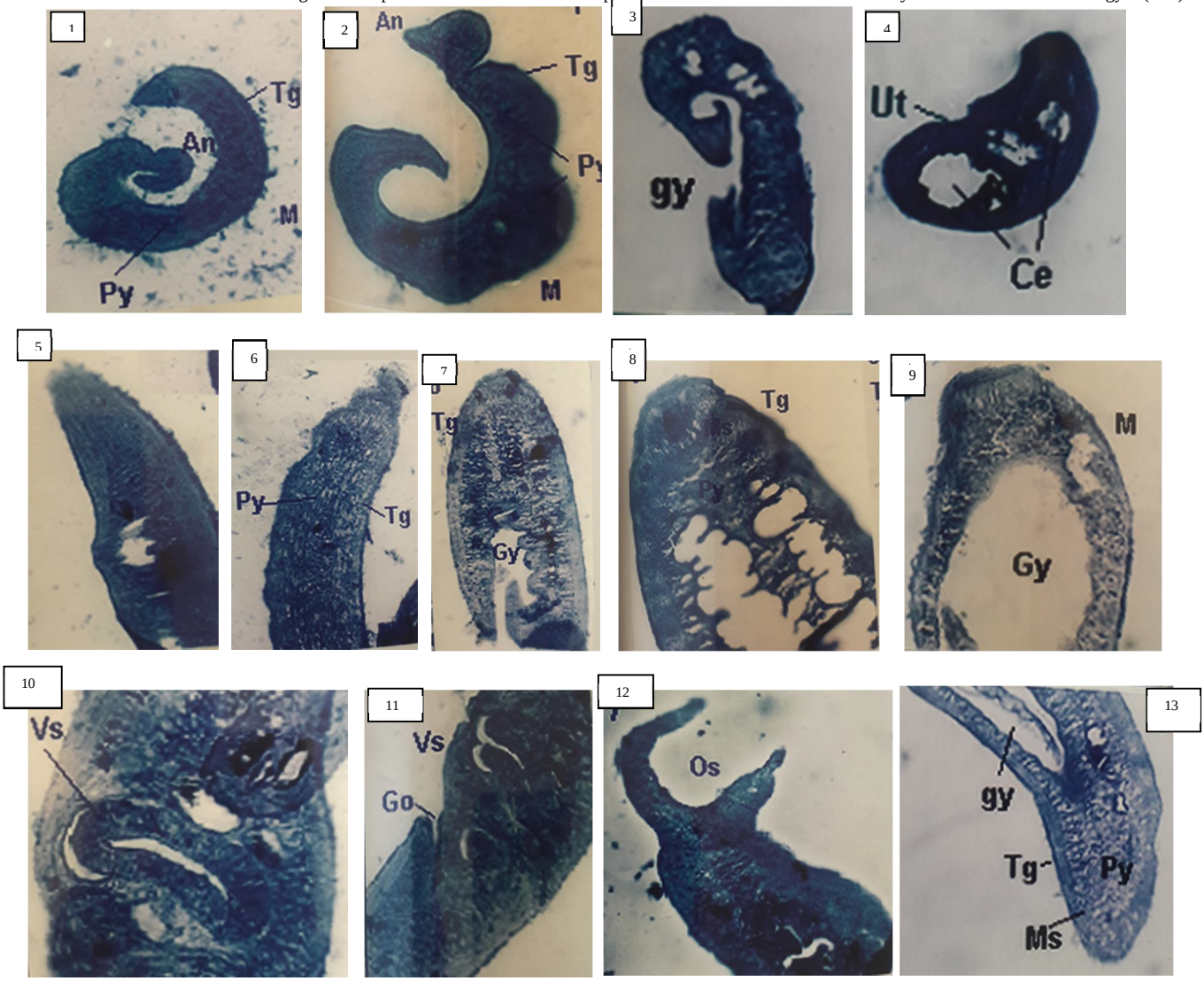
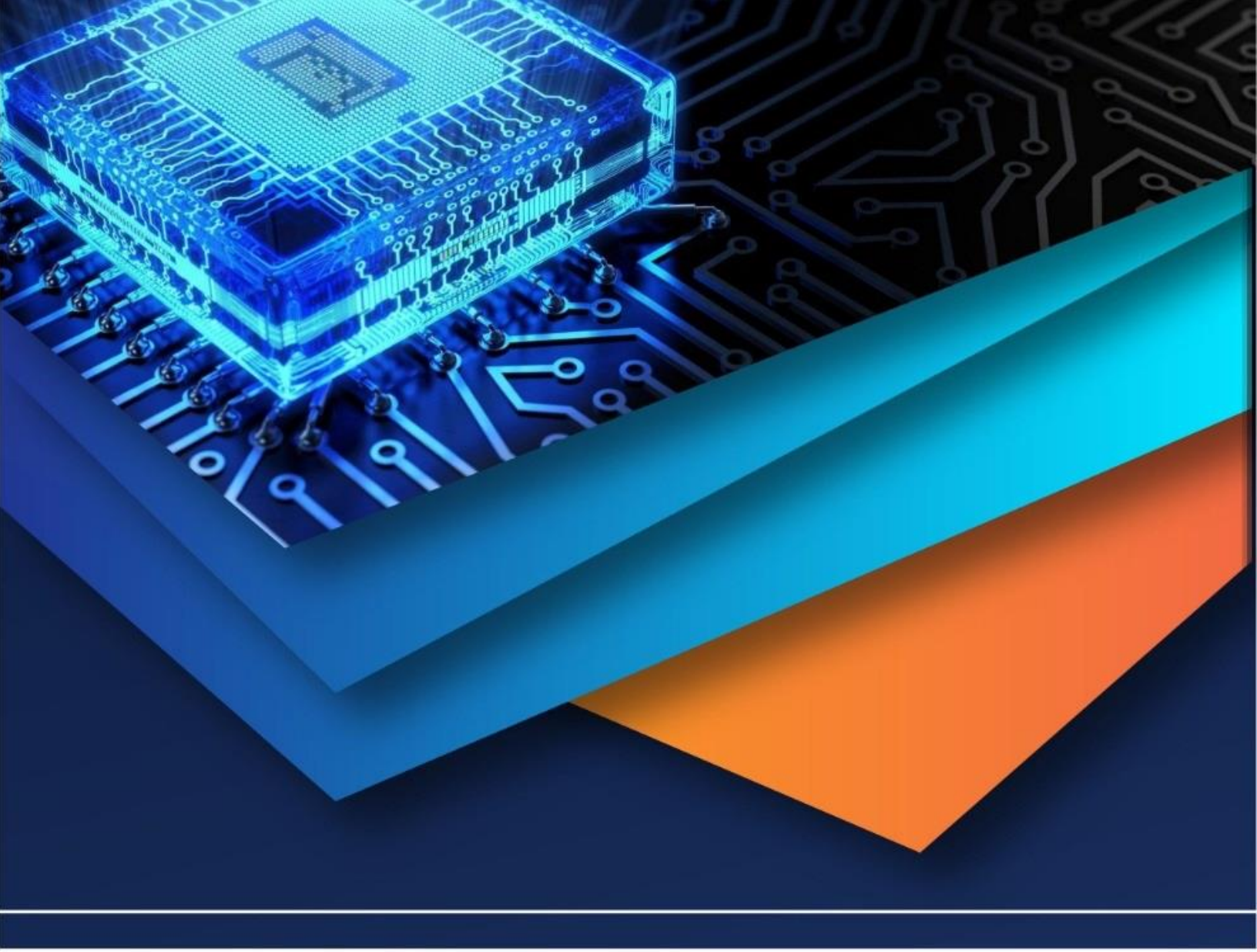


Fig.1 , 2. LDH activity in coiled male S.spindale. Fig.3. LDH activity in section of male S.spindale. Fig. 4. Transverse section of the female S.spindale showing the LDH activitiy. Fig.5. Male S.spindale showing the LDH acitivity of the musculature of the body wall. Fig. 6 LDH activity shown in the body wall of the anterior end of the male S.spindale. Fig. 7. LDH activity shown in the section of S.spindale. 8. Magnified view of the section of S. spindale showing LDH activity. Fig. 9. LDH activity shown in the male S.spindale. Fig. 10. Magnified view of the Ventral sucker of the male S.spindale shoing LDH activity. Fig. 11. Male S. spindale showing LDH activity in the gynaecophoric canal and the ventral sucker. Fig 12. LDH activity in the oral sucker of male S.spindale. Fig 13. Section of the male S.spindale showing LDH activity in the muscular of body wall and parenchyma.(Labellings are An-Anterior end, M-male, F-Female, Tg-Tegument, Ms-Musculature,Gy/Go-Gynaecophoric canal, Vs-Ventral sucker, Os-Oral sucker, py-parenchyma)



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