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HISTOCHEMICAL LOCALIZATION OF SUCCINIC DEHYDROGENASE IN SCHISTOSOMA SPINDALE

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Abstract: *Succinic dehydrogenase(SDH) is an essential enzyme of tricarboxylic acid cycle bound to inner surface ofthe inner mitochondrial membrane. The most important activity of this enzyme is its ability to transfer electrons to the respiratory chain. Schistosoma spindale, the blood fluke, is a parasite inhabiting the portal and mesenteric veins of Bubalus bubalis. Indoplanorbis exustus is the intermediate host of S. spindale. Blood plays a key role in the regulatory activity of host cellular processes. When the parasite moves in the host environment, it is carried by respiratory currents or by the flow of blood stream. The passage of the parasite through blood and the host tissues is a risky one as the parasite is exposed to unfavourable pH, attack by macrophages and antibodies. SDH is an enzyme helping the parasite in leading the life cycle in the blood vessels of the host. SDH activity localized in the cryostat section of the S.spindale, seen as purplish violet diformazon granules in the various tissues of the parasite determines the enzyme activity. The present study depicts the histochemical localization of SDH activity found in the cryostat sections of S.spindale.*

Keywords*: Succinic Dehydrogenase(SDH),Schistosoma spindale,Bubalus bubalis, tricarboxylic acid cycle.*

1. INTRODUCTION:

Succinic dehydrogenase is an essential enzyme of tricarboxylic acid(TCA) cycle. It is also one of the flavoproteins in which flavin is covalently linked to the protein. It transfers electrons to the respiratory chain which is its important ability. It is the only dehydrogenase in the TCA cycle which involves the direct transfer of hydrogen atoms from substrate to a flavoprotein without the participation of NAD^+. SDH catalyses the formation of fumarate by removal of one hydrogen atom from each α-carbon atom of succinate. Bueding(1962) reported that in parasitic helminths, succinate dehydrogenase acitivity shows directing the fumarate reduction rather than succinate oxidation[1]. Succinic dehydrogenase serves as an electon acceptor for succinate and as an electron donor for fumarate depending on physiological environment of parasite. According to Kmetec and Bueding(1961), Cytochromes and succinic dehydrogenase were relatively associated to form succinic oxidase. *S.spindale* is a blood fluke inhabiting the host *B.bubalis*[2]. Histochemical techniques are widely used for localization of specific enzymes for the study in sections.The role of SDH activity is very significant in parasitic helminths. Histochemical studies on *S. spindale* related to the SDH activity confirm its important role in the adaptation of the parasite to the anaerobic ambience.

2. LITERATURE REVIEW:

The literature review highlights some of the crucial aspects of the parasitic mode of life related to the helminth, *S.spindale*. Succinate Dehydrogenase (SDH) is part of both the citric acid cycle and respiratory electron transfer chain and it consists of four subunits(named A to D) encoded by the nuclear genome [3]. The succinate dehydrogenase of adult *F.hepatica* was found to exist in active and inactive forms. The activation of the enzyme by succinate followed first-order kinetics. The extent of activation of *F.hepatica* succinate dehydrogenase depended on the nature and concentration of the activator, pH and the temperature[4]. Mrunalini,1998 had localized histochemically SDH activity in the acanthocphalan, *Moniliformis dubius* and found SDH activity to participate actively in a functionally operative TCA cycle[5].Succinic dehydrogenase in parasitic helminths has a high affinity for fumarate and low affinity for succinate[6]. Zenka et al.(1987) found a low proportion of fumarate reductase suggesting the existence of aerobic processes in helminths[7]. There are several examples of succinate dehydrogenases that are essential for virulence and can be discriminated for drug targeting. Salmonella enterica encodes an annotated succinic dehydrogenase and fumarate reductase(8,9). SDH activity of an organism in the host is important for its viability in host tissues. SDH served as a potential Biomarker for targeted drug development in parasitic infestation like Hymenolepiasis [10]. Parvathi and Aruna(2011) have found the correlation of hyperglycemia and succinate dehydrogenase activity during hymenolepiasis in mice and treatment with praziquantel. [11]. Histochemical demonstration of proteins was carried out in the parasite *S.spindale* by Vanita et al.(2018,12). The activity of lactate dehydrogenase was histochemically localized in the sections

of *S.spindale* (Vanita et al.2019,13). Succinic Dehydrogenase activity plays an important enzyme of the parasitic adaptation of life in the host

3. MATERIALS AND METHODS:

Histoenzymological Studies (Succinate Dehydrogenase) 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 usedto fix the cryostat sections 12-16 µ thick. These were put in incubating medium for 30 minutes to 1 hour at 37ºC. The sections were immersed in 10% formalsaline 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. Purple formazon deposits(NBT) were formed at the sites of enzyme activity[14].

4. RESULT:

The Succinic Dehydrogenaseactivity localized in the cryostat sections of the parasite were seen as purplish violet diformazoan granules in the different regions of the parasite. The intensity of the colour in the various tissues of the parasite determines the Succinic dehydrogenase activity. The distribution of SDH activity in the various regions as follows:

- The distribution of the enzymatic activity was very conspicuously seen in the different layers of the bodywall. The SDH activity was more pronounced in the tegument(Fig.1,2,3,4) while the muscular layer, circular muscular layer showed moderate enzymatic activity(Fig.1,2,3,4,5). Significant amount of Succinic dehdrogenasee activity was seen in the parenchyma(Fig. 1,2,3,4,5).
- The SDH activity varied from weak to moderate in the different regions of the sections of the male and the female *S. spindale*(Fig.1,3,4,5). The activity was found to be denser in the section of female when compared to that of male *S. spindale*(Fig.4,5,6,7,8,9). The sections of the female depicts dark blood contents in the caeca. As the parasite is sanguivorous and has a prominent role in sucking the host' blood. The caeca of the female parasite are found to be filled with haemolyse fluid(Fig.6,7,8)
- The oral sucker and the ventral sucker showed a denser enzyme activity.(Fig.12,13). The muscular layer of the ventral sucker showed profuse activity of SDH(Fig.12,13).
- Remarkable SDH activity was seen in the uterus and the egg. The horns of the egg were evidently seen in the section.(Fig.10,11).

5. ANALYSIS & DISCUSSION:

SDH activity, profoundly found in the bodywall, indicates the role of the musculature of the bodywall which is involved in movement of the parasite. Reissenweber et al. 1976, studied the activity of SDH in *Echinococcus granulosus* and found the subtegumental and flame cells were rich in Succinic Dehydrogenase enzyme[15]. The SDH activity was found to be present in the tegument, subtegumental cells and intestinal epithelium as per the studies made by Panitz(1968) in *Fasciola hepatica*[16]. A quantitative histochemical study was carried out on axial musculature of *Noemacheilus barbatulus,L*. by Kilarski and Kozlowsha,1985[17]. On the basis of succinate dehydrogenase (SDH) and myofibrillar ATP-ase activity, 5 types of muscle fibers are described. When the SDH method was used, red, tonic, intermediate, and white muscle fibers were easily observed.. Barry and Malone, 1968 studied the enzyme histochemistry of the adult liver fluke, *F. hepatica* and found SDH activity to be present[18]. In the present study, the SDH activity varied from weak to moderate the different regions of the sections of the male and the female *S. spindale*. The activity of was found to be denser in the section of female when compared to that of male *S. spindale*. Coles, 1973 had reported reduced tricarboxylic acid cycle enzyme activities in paired adult S. *mansoni* and *S. japonicum* in camparison to those reported for *S. mansoni* cercariae[19]. Smith and Brown(1977) have found the SDH activity more than twice in paired adult *S. japonicum* than in paired adult *S. mansoni*[20] The SDH activity was found low in both species of adult worms. The tricarboxylic acid cycle enzymes activities in adult *S. mansoni* and *S.japonicum* were low. Smith and Brown(1977) have further substantiated that anaerobic glycolysis is the major energy source in adults of both species[20]. In the present study, profuse SDH activity found in the suckers reveal the fact that suckers are regions having a functional role in the intake of food, nutrients and adhesion to the blood vessel. The suckers are the regions always in action engulfing blood and serving as an adhesive organ. In the present work carried out notable SDH activity was seen in the egg. The SDH activity was studied histochemically, in *F. hepatica* by Panitz, 1968 and SDH activity was found to be localized in acetabulum, oral sucker, cirrus, cirrus pouch, prostrate glands, sperm, pouch wall, oviduct and uterus[16]. Barret(1977) has provided evidence of a functionally operative TCA cycle in the trematode, *S. mansoni*[21]. The significance of these studies and the present study indicate the regulation of the SDH, a tricarboxylic acid cycle in parasitic helminths.

CONCLUSION:

Energy production is the main function of catabolic pathways. This energy is usually in the form of ATP which is required for mechanical synthetic or osmotic work. Citric acid cycle is the major metabolic pathway for the production of energy in all animal tissues. Anaerobic succinate production often depends on the reversal of the last Krebs cycle reaction(Von Brand,1973,22). The major function and significance of the citric acid cycle is much greater than that of mere oxidation of carbohydrates, lipids and proteins(Murray et al.1993,23). and essentially at this point, where the carbohydrates, proteins and lipids meet(Smyth, 1969,24). The carbondioxide production could be restored not only by the C_4 dicarboxylic acid (succinic, fumarate, malate and oxaloacetate) but also by C_5 compound α - oxoglutarate and by the C_6 tricarboxylic acids, citrate, aconitrate and isocitrate. Enzyme present in the tissues could catalyse the conversion of citrate to aconitate and isocitrate to α-oxoglutarate by a similar oxidative decarboxylation gets converted to succinate. Inspite of intensive experimentation, how Schistosomes obtain the bulk of its energy is still ambiguous. Information related to the metabolism of Schistosomes species infecting animals is not sufficient, probably on account of their general unavailability and difficulty of maintaining them in the laboratory. Therefore in the present study, the author has chosen to study the major TCA cycle enzymes i.e. Succinic Dehydrogenase in the *S.spindale*. Histochemical findings in the present study substantiate the role of succinate dehydrogenase for the life in the host's blood.

REFERENCES:

1. Bueding, E. (1962). Comparative aspects of carbohydrate metabolism. Proc. Fedn Am. Socs. *Exp. Biol*., 21: 1039-1046.
2. Kmetec, E. and Bueding, E. (1961). Succinic and reduced diphosphopyridine nucleotide oxidase systems of Ascaris muscle. *J. Bio. Chem*. 236:584-591.
3. Barrett,J.(1978). Activation of succinic dehydrogenase from adult *Fasciola hepatica* (Trematoda). *Parasitology*.76 (3):269-75.
4. Mrunalini(1998). Histochemical and Histoenzymological studies on the acanthocephalan parasite *Moniliformis dubius*, parasitic in the house rat *Rattus rattus rufescens.* Ph.D Thesis. Osmania University. Hyderabad, India.
5. Zenka, J., Prokopic, J. and Zeybek, H.(1987). Malic enzyme, malate dehydrogenase, fumarate reductase qand succinicate dehydrogenase in the larvae of *Taenia crassiceps*(Zedar,1800). *Trop. Geography Med*. 38(1):47-54.
6. McClelland, M., Sanderson, K.E., Spieth, J., Clifton, S.W., Latreille, P. (2001). Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. *Nature* 413:852-856.
7. Spector, M.P., Garcia del Portillo, F., Bearson,S.M., Mahmud, A.,Magut, M., Finlay, B.B.,Dougan, G., Foster, J.W. and Pallen, M.J.(1999). The *rpo*S-dependent starvation-stress response locus *sti*A encodes a nitrate reductase (*nar*ZYWV) required for carbon-starvation-inducible thermotolerance and acid tolerance in *Salmonella typhimurium*. *Microbiology*. 145, 3035-3045.
8. Parvathi, J. and Aruna K.(2012). Succinate Dehydrogenase and Eosinophils as Biomarkers of Hymenolepiasis. *Cibtech J Zool*. Vol 1(1),8-13. *An International Online Journal.*
9. Parvathi, J. and Aruna, K. (2011b). Correlation of hyperglycemia and succinate dehydrogenase activity during hymenolepiasis in mice and treatment with praziquantel. *IOSR J. Pharmacy.* 1(1): 23-30.
10. Vanita, M., Aruna, K and Parvathi, J.(2018). Histochemical localization of Proteins in *S. spindale*. *Journal of Current Science.* 19(9):1-7.
11. Vanita, M., Aruna, K and Parvathi, J.(2019). Histochemical localization of Lactate Dehydrogenase in *S.spindale. International Journal for Research in Applied Science and Englineering Technology*. 7(1):696-699
12. Reissenweber,N.J., Vercelli-Retta,J., Siri,A.M., Lozano,W. (1976). Histochemistry and histoenzymology of the hydatid cyst(*Echinococcus granulosus* Batsch, 1786)-II. Scolices and brood capsules. *Zeitschrift fur Parasitenkunde*, 48(1)25-33.
13. Panitz, E.(1968). Histochemical Study of some enzymes of the common liver fluke, *Fasciola Hepatica* L.Ph.D. Thesis. Oregon State University. Corvallis, Oregon, United States of America.
14. Kilarski, W and Kozlowsha, M. (1985). Histochemical and electronmicroscopical analysis of muscle fibre in myotomes of teleost fish(*Noemacheilus barbatulus L.*). *Gegenbaurs Morphologisches Jahrbuch*, 131(1):55-72.
15. Barry, D.H. ,Mawdesley-Thomas, L.E. and Malone, J.C. (1968). Enzyme histochemistry of the adult liver fluke, *Fasciola hepatica*. *Experimental Parasitology*. 23(3).355-360.
16. Smith, T., M. and Brown, J. N.(1977). Tricarboxylic acid cycle enzyme activities in adult Schistosoma mansoni and *Schistosoma japonicum. Transactions of the Royal Society of Tropical Medicine and Hygiene, d University Press.*71(4):329-330.
17. Barrett,J.(1977). Energy metabolism and infection. *Symposia of the British Society for Parasitology*, 15:121-144.
18. Rosenberg, R.N. and Pascual, J.N. (2015). Rosenberg's Molecular and Genetic basis of Neurological and

Psychiatric disease. Fifth Edition. Academic Press. United States of America.
19. Barrett, J. (1981 a). Biochemistry of Parasitic Helminths. McMilan Pub. Ltd. U.K. pp.130.
20. Pearse, A.G.E.(1968). Histochemistry . Theorotical and applied. Vol.I 3rd Ed. Little Brown, Boston.
21. Coles, G.C.(1973a).The metabolism of Schistosomes. A review. *International Journal of Biochemistry*, 4:319-337.
22. Von Brand, T.(1973. "Biochemistry of paasites". 2nd Ed., Academic Press, New York.
23. Murray, R.K., Granner D. K., Mayer, P.A. and Rodwell, V.W.(1993). Harpers Biochemistry 23rd Ed. Prentice Hall International, Inc. Englewood Cliffs, New Jersey, P.806.
24. Smyth, J.D.(1969). "The physiology of trematodes".2nd edition. Cambridge University Press.

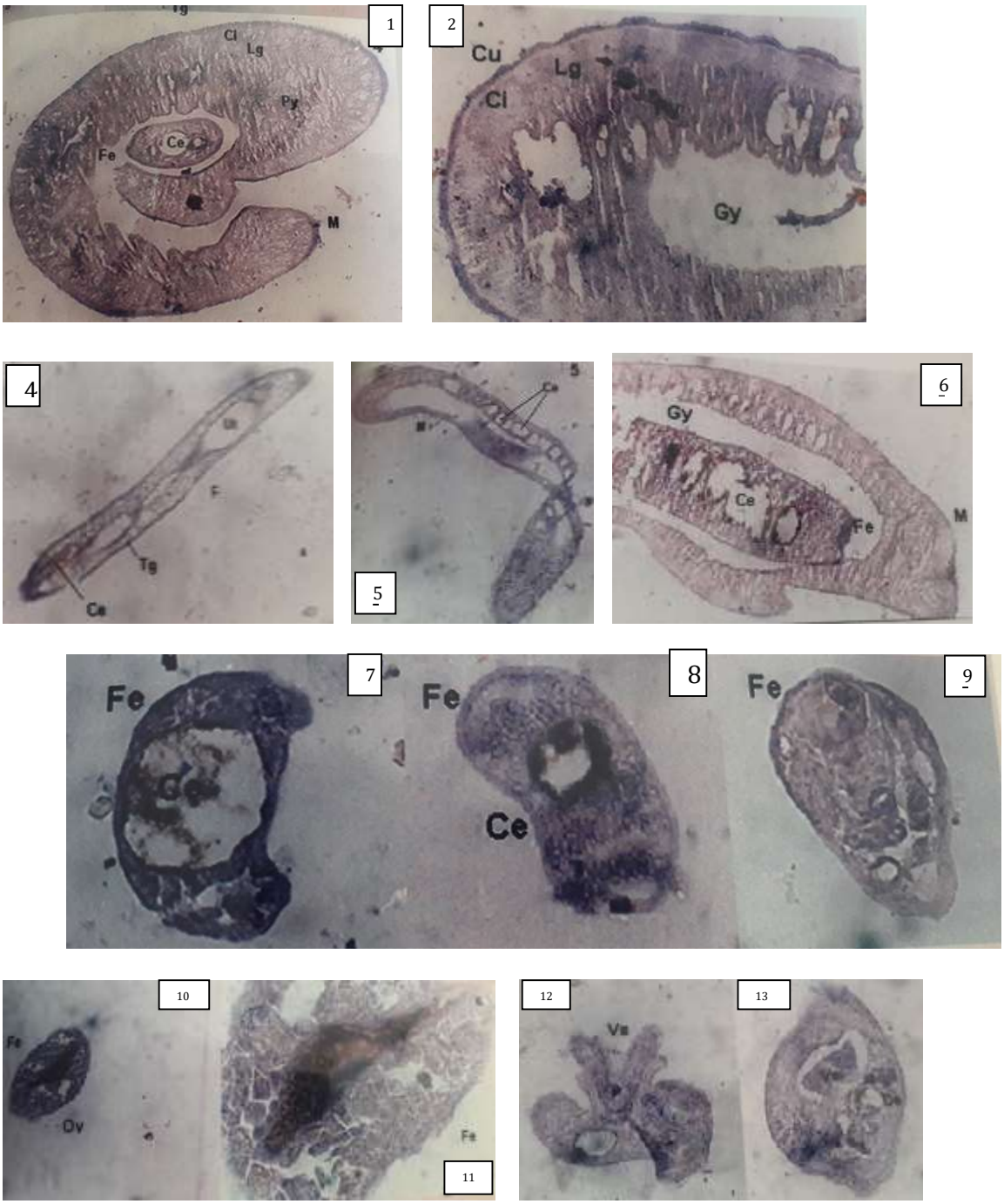

Fig. 1 SDH activity in the section of male and female *S. spindale*. Fig. 2 SDH activity in the bodywall of *S.spindale*. Fig.3, 4 body wall of female *S. spindale*. Fig. 5 SDH activity shown in the body wall of the male and the female *S. spindale*. Fig. 6,7,8,9 SDH activity in the female *S. spindale*. Fig.10,11 SDH activity of the female *S.spindale* and showing the centrally placed *spindale* (hat)shaped egg. Fig. 12,13. SDH activity shown in the Ventral sucker.