Cibtech Journal of Zoology ISSN: 2319–3883 (Online)
An Online International Journal Available at http://www.cibtech.org/cjz.htm
2012 Vol. 1 (1) May-August, pp.8-13/Parvathi and Aruna
Research Article

SUCCINATE DEHYDROGENASE AND EOSINOPHILS AS BIOMARKERS OF HYMENOLEPIASIS

J Parvathi[1] and K Aruna[2]
[1]Faculty @ help BIOTECH Academy, #201, BhavaniShri Sheela Avenue, Krishna nagar, Musheerabad, Secunderabad, A.P., India
[2]PG Department of Zoology, Vivek Vardhini College, Jambagh, Hyderabad-500095, A.P., India
**Author for Correspondence*

ABSTRACT
Disease producing organisms require a highly specialized environment for their growth and propagation, usually found only in the tissues of man or other animals. Although the morbidity and mortality due to such infections is not alarming, they adversely affect the general health and productivity of adults, mental and physical growth of children, especially those suffering from malnutrition. Hymenolepis nana (dwarf tapeworm) is one such common cosmopolitan intestinal parasite of man and mouse, which can persist in a single host for many years, leading to even crowding effect, traumatizing the host further. As a cestode helminth, the hallmark of its infection is eosinophilia, a common biomarker of helminthiasis. But succinate dehydrogenase, a key enzyme of the energy generating kreb's cycle, at the succinate-fumarate junction is also a potential biomarker, since; kreb's cycle is bi-directional in helminthes and uni-directional in the host. The excess of succinate is released by the parasite into the host, later to be absorbed by the host. In the present investigation, a quantitative increase in eosinophils and a qualitative increase in succinate dehydrogenase activity (demonstrated histochemically), have been observed. Thus, any deviations at this junction of this energy cycle, mediated by succinate dehydrogenase can be successfully interpreted for combating the parasitic attack. Hence eosinophils and succinate dehydrogenase levels in a traumatized host can be used as effective biomarkers, and utilized for the development of specific chemotherapy and vaccines.

Key Words: *Eosinophils, Succinate Dehydrogenase, Hymenolepiasis, Helminthes, Biomarkers*

INTRODUCTION
Parasitic diseases seldom appear in the shape of explosive epidemics and as such do not dramatically shoot-up to the banner headlines of newspapers. But, this is more than compensated by the relentlessly corrosive design of many a parasitic disease which though not killing, inflicts mass morbidity in a vast segment of human population, exacting a devastating economic toll in endemic areas. It is only obvious that research on parasitic disease has not received until 1980's, its legitimate share of importance, emphasis and patronage from the scientific world, national and international (Chowdhary, 1978).
The probability that two organisms will establish a host-parasite relationship depends largely on various factors. It is necessary to examine the nature of responsiveness in the components when host and parasite exist as a single biological entity (Owen, 1972).
Helminthiasis is caused by the invasion of human and animal body by the parasitic worms belonging to the class nematoda (roundworms), cestoda (tapeworms) and trematoda (flukes). Hymenolepis nana, the Dwarf tapeworm, is a cosmopolitan intestinal cestode common to man and mouse, especially in the tropics and the subtropics (Belding, 1965). This is the only known cestode transmitted directly (Cheng, 1964) from one definitive host to another.
The invasion of a host by a helminth is usually accompanied by some mechanical damage to the tissues (Parvathi and Aruna, 2010). The amount of trauma is often dependent upon the tissue invaded and on the number of parasites involved by the following means: mechanical trauma; obstruction of hollow viscera and circulatory channels; utilization of substances normally destined for body nutrition; depletion of the blood; chemical intoxication; and occupation of space and displacement of tissues (Sumner, 1988).

Haematological studies help in evaluating the response of different types of blood cells in conditions of physiological stress, especially due to parasitism. The primary pathological consequence of hymenolepiasis, like any other helminthiasis is eosinophilia. The dynamics of this response have been particularly well studied in murine hosts. Eosinophil and plasma cells are also predominant in the infected tissues of mice infected with tapeworms such as *Hymenolepis microstoma* (Lumsden, 1975b).

In helminthiasis not only the histology and haematolgy of the host gets traumatized, but marked histochemical alterations too have been reported by various authors including the tissue substrates like the carbohydrates, proteins and lipids and various enzymes related to their metabolism(Parvathi, and Aruna, 2011b). Succinate dehydrogenase is one such key enzyme of the energy generating Kreb's cycle mediating the interconversion of fumarate↔succinate. Succinate dehydrogenase(SDH) activity has been studied in rat liver infected with *A.ceylanicum* and *N.braziliensis* (Singh *et al.,* 1992). The most important activity of SDH is its ability to transfer electrons to the respiratory chain by the formation of fumarate by removal of one hydrogen atom from each α-carbon of succinate. SDH in mammalian tissues has high affinity for succinate oxidation and a low affinity for fumarate reduction (Schiebell, and Saz, 1966). In contrast, SDH in parasitic helminths has high affinity for furmarate than succinate (Barrett, 1981a, b). Moreover, since Kreb's cycle in bidirectional in helminthes, the excess succinate generated by the parasite is absorbed by the host to produce more energy to cope-up the trauma.

Hence, Eosoninophil count and succinate dehydrogenase activity can be successfully used as Biomarkers for assessment of the extent of parasitism. This can open new vistas for further research in more effective targeted chemotherapy with the application of biotechnology.

MATERIALS AND METHODS

Adult healthy male Swiss albino mice, *Mus musculus* about 4-5 weeks old, weighing 20-25 grams, maintained under good laboratory practices (GLP) conditions (Bodil, 1994), were divided into two batches. One batch was the uninfected control batch. The other batch was orally infected with about 100 viable eggs of *Hymenolepis nana* per mouse, and maintained as the infected batch. These infected mice were thus maintained until the 16^{th} day of infection to complete the life cycle of the parasite. These two batches were sacrificed at appropriate times, by decapitation. Blood was analyzed for eosinophils.

5µ thick unfixed cryostat sections of the host intestine were stained with nitroblue tetrazolium method for the histochemical localization of succinate dehydrogenase activity and microphotographed, at 40x magnification. The dehydrogenases of the tricarboxylic cycle are redemonstrable histochemically by the tetrazolium-formazan reduction reaction (Lillie, 1965). The reduction of the colourless tetrazolium salt, by the hydrogen of the substrate, produces an intensely coloured water-insoluble formazan deposit, which can be seen at the site of enzyme activity (Pearse, 1954; Roberts, and Lucchese, 1955; Tsou *et al.,* 1956). The procedure followed in this study is the general histochemical staining method for dehydrogenases (Bancroft, 1966).

Some part of the intestine was processed for scanning electron microscopy (Bozzola, and Russell, 1999), to elucidate the mechanical trauma in the traumatized murine host, at Ruska laboratories, Rajendranagar, Hyderabad.

RESULTS AND DISCUSSION

The scanning electron micrographs of mouse intestine infected with Hymenolepis nana are shown in Figures 1 (A to C). Large number of worms in the intestinal wall disrupting more than one site of the host is shown in A. A magnified image of the worm penetrating the host villus has been shown in B. Many perforations in the intestinal wall are seen in C.

The histochemical localization of succinate dehydrogenase enzyme activity in host tissues is depicted in Figures 2A and B. In the uninfected mice is shown in A. The same in *Hymenolepis nana* infected host is localized in B, which clearly shows an increased intensity in the colored formazan deposits. The increased color is directly proportional to the increased enzyme activity.

Eosinophil count (E): The variations in control and *Hymenolepis nana* infected *Mus musculus* are
Control : 0%
Infected : 4%
The eosinophils have made their appearance in the infected host blood indicating increased immune response in the host. These results are statistically tabulated in Table 1.

Table 1: Comparison of Eosinophil count during Hymenolepis nanai infection with the uninfected control host Mus musculus.

Parameter	Batch	Mean ± S.D.	't' Value	% Change
Eosinophils [a]	Control	-- -- -----	*	---------
(E)	Infected	4 ± 0.707	*****	---------

The values are mean of five individual observations.
± indicates standard deviation.
*The values show statistical significance, *, at p < 0.05; non-significant (NS) at p > 0.05.*
[a] non- parametric mann-whitney rank sum test applied.

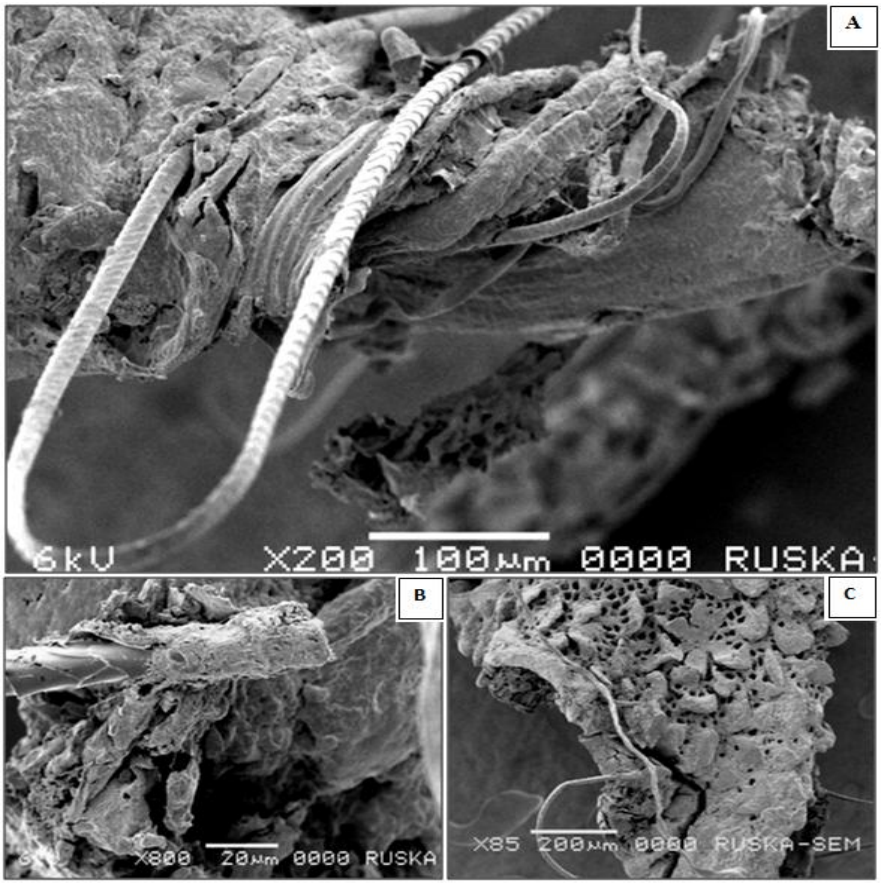

Figure 1: Scanning Electron Micrographs of Hymenolepis nana Infected Intestine of Host Mus musculus. A - Many worms perforating the host intestine. B - A single worm penetrating intestinal villus. C - Perforations seen in the host intestine**.**

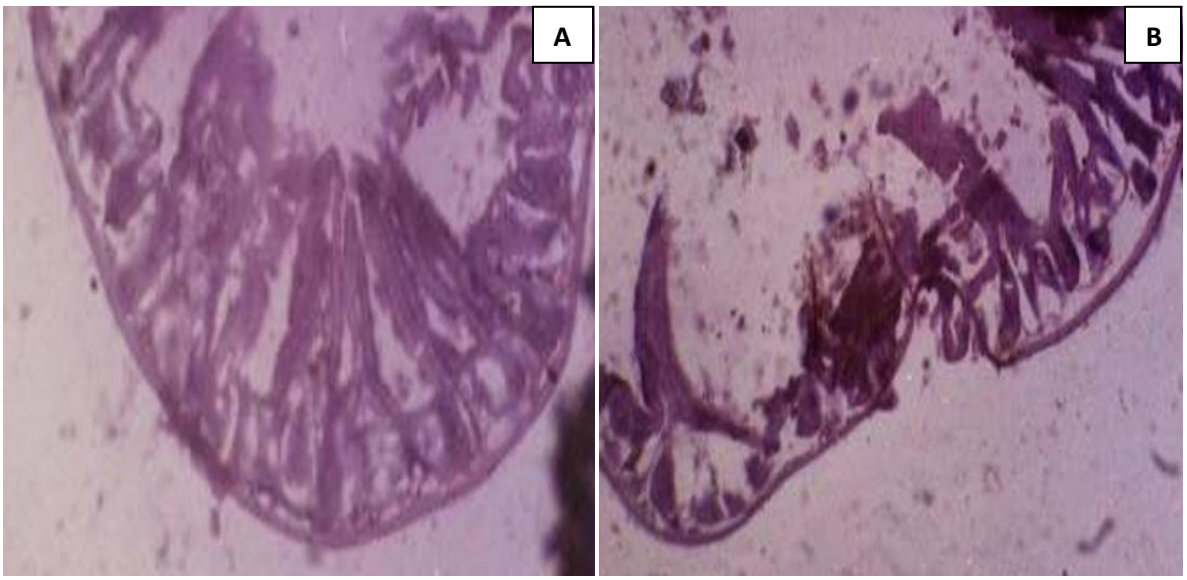

Figure 2: Nitroblue tetrazolium stained Mus musculus intestine sections for histochemical demonstration of succinate dehydrogenase enzyme activity during hymenolepiasis. A - Uninfected control host intestine section showing succinate dehydrogenase activity. B - Intestine section of the host infected with Hymenolepis nana, showing increased succinate dehydrogenase activity proportional to the increased colour intensity. H.nana can also be seen as a ladder like structure.

Hymenolepis nana inhabits the ileum part of host's small intestine and it is in this region that the actual extent of damage that the worm can inflict mechanically is visualized clearly through the scanning electron micrographs. The infected host tissue investigated, reveals the mechanical injury at the site of the habitat of this cestode. The host's tissue is disrupted, the worm bores into the intestine leading to the leakage of various host's substrates, enzymes and blood and also a lot of tissue debris into the lumen of the intestine (Innes, 1967). The crowding effect (Heyneman, 1953) due to recurrent auto-infections in the immuno-compromised hosts leads to many more complications in the host like pronounced intestinal disturbances, retarded growth and weight loss and chronic inflammation (Simmons *et al.,* 1967). In the warmer countries in populations with poor sanitation, this crowding effect could prove to be a major health problem and even hazardous, especially among children (Chero *et al.,* 2007).

Blood is one of the most important specimens studied in various sections of the laboratory in search of blood related alterations during infections and diseases. Accordingly, haematology can be used as clinical tool for the investigations of physiological and metabolic alterations. Eosinophils count of an animal has close relationship with many disorders and diseases. Increased eosinophil count is often associated with parasitic infection; allergic reaction, and certain leukemias. It is also the hallmark of helminth infection (Murrell, 1982). The studies on murine haematology due to helminthiasis also causes drastic alterations in the white blood cells in general and specifically in its various components like neutrophils, lymphocytes etc. All these various imbalances caused by helminthiasis demonstrate the extent of damage that a particular helminth worm can incur in its host (Parvathi, and Aruna, 2011a). In the present investigation, the appearance of eosinophils in the infected host blood depicts the increased antibody-dependent cytotoxic defence of the host against H.nana infection.

In all the animal tissues, energy is produced in the form of adenosine triphosphate through the major metabolic pathway of the Tricarboxylic acid (TCA) cycle or citric acid cycle or Kreb's cycle. Many infected helminths are facultative aerobes. All the Kreb's cycle enzymes are found in helminths. Kreb's cycle provides a source of mitochondrial power for the reduction of fumarate to succinate.

In the present work, it is found that the host infected intestine showed an increase in color intensity when stained for SDH activity. Intestine being the habitat of the parasite Hymenolepis nana, it is an established fact in helminths in general that succinate formation is bi-directional (Kreb's cycle and reverse Kreb's cycle) which is then excreted by the worm into its surroundings. This excreted helminth succinate could then be absorbed and utilized by the host (Chappell, 1980). The absorbed succinate would then be converted to fumarate to ultimately complete the Kreb's cycle via malate and regeneration of oxaloacetate to yield the increased energy demands of the traumatized host intestinal tissue in combating pathogenesis. Hence either of the increased interconversions of succinate and fumarate would result in an increased succinate dehydrogenase activity.

Hence during Hymenolepis nana infection of any helminth infection in general, Eosinophil count and Succinate dehydrogenase enzyme activity can be effectively used as Biomarkers to assess the severity of infection. The quantitative assay of these two can be effectively extrapolated for the development of targeted chemotherapy and vaccines. This is more advantageous in the developing countries where there is need for low-cost, single-dosage drugs administered orally without specific diet requirements.

ACKNOWLEDGEMENTS

We are grateful to the Management of Vivek Vardhini Educational Society, for providing us the Laboratory facilities at the P.G. Department of Zoology. We are also grateful to the Ruska laboratories, Rajendra Nagar, Hyderabad, for the Scanning electron microscopy work.

REFERENCES

Chowdhary AB (1978). The problem and prospect of chemotheraphy in parasitic diseases. The Symp. on Clin. Pharmaco-Kinetics and Guest lecture on the problem and prospect of chemotheraphy in parasitic diseases. *Proceedings of the V Southern Regional Conference of Indian Pharmacology Society* 45-46.

Owen D (1972). Common parasites of laboratory rodents and lagomorphs. *Medical Research Council*, Laboratory Animals Centre Handbook No. 1.

Belding DL (1965). Textbook of Parasitology. New York: *Appleton-Century-Crafts* 1374.

Cheng TC (1964). The biology of animal parasites. Philadelphia: WB Saunders 727.

Parvathi J and Aruna K (2010). Histopathological assay of induced hymenolepiasis in Mus musulus and restoration of normalcy with praziquantel. *The Bioscan* **5**(4) 661-664.

Sumner BEH (1988). Basic Histochemistry. John Wiley and Sons.

Lumsden RD (1975b). Surface ultra structure and cytochemistry of parasitic helminths. *Experimental Parasitology* **37** 267-339.

Parvathi J and Aruna K (2011b). Correlation of hyperglycemia and succinate dehydrogenase activity during hymenolepiasis in mice and treatment with praziquantel. *IOSR Journal Pharmacy* **1**(1) Available at: http://www.iosrphr.org/pages/vol1%20issue1.html.

Singh SP, Katiyar JC and Srivastava VML (1992). The enzymes of the tricarboxylic acid cycle in Ancylostoma ceylanicum and Nippostrongylus brasiliensis. *Journal of Parasitology* **78**(1) 24-29.

Schiebell W and Saz HJ (1966). The pathway for anaerobic carbohydrate dissimilation in Hymenolepis diminuta. *Comp Biochemistry Physiology* **18** 151-162.

Barrett J (1981a). Biochemistry of parasitic helminthes. London: McMillan Publ Ltd. 283-286.

Barrett J (1981b). Biochemistry of parasitic helminthes. London: McMillan Publ Ltd. 130.

Bodil L (1994). Good Laboratory Practice, Handbook of Laboratory Animal Science: *Selection and Handling of animals in Biomedical Research* (London, NewYork) CRC Press **1** 37-40.

Lillie RD (1965). Histopathologic technic and practical histochemistry. Mc Graw-Hill.

Pearse AGE (1954). Application of the alkaline tetrazolium reaction to the study of reducing groups in tissue sections. *Journal of Pathology and Bacteriology* **67** 129.

Roberts LW and Lucchese G (1955). Sulfhydryl localization and tetrazolium reduction. Reversible inhibition of its reduction by N-ethyl maleimide. *Stain Technology* 30 291.

Tsou KC, Chang CS, Nachlas MM and Seligman AM (1956). Synthesis of some ρ-nitrophenyl substituted tetrazolium salts as electron acceptors for the demonstration of dehydrogenases. *Journal Amer Chemistry Sociology* **78** 6139.
Bancroft JD (1966). Enzyme histochemistry. In: *Theory and Practice of Histological Techniques*, edited by Bancroft JD and Stevens. *Churchill Livingstone* 407.
Bozzola JJ and Russell LD (1999). Electron Microscopy: Principles and Techniques for Biologists. *Massachussets: Jones and Barlett Publication* 2.
Innes JRM (1967). In: *Pathology of Laboratory Rats and Mice*, edited by Cotchin E, Roe FJC (*Oxford: Blackwell Science Publishers*) **21**.
Heyneman DJ (1953). Auto-reinfection in white mice resulting from infection by Hymenolepis nana. *Journal Parasitology* **39**(*Suppl*) 28.
Simmons ML, Richter CB, Franklin JA and Tennant RW (1967). Prevention of infectious diseases in experimental mice. *Proceedings of the Society for Experimental Biology and Medicine* **126** 830-837.
Chero JC, Saito M, Bustos JA, Blanco EM, Gonzalvez G and Garcia HH (2007). Hymenolepis nana infection: symptoms and response to nitazoxanide in field conditions. *Trans R Soc Trop Med Hyg.* **101**(2). Available at: http://www.tropicalmedandhygienejrnl.net/article/S0035-9203%2806%2900140-4/abstract
Murrell KD (1982). Helminths. In: The Mouse in Biomedical Research. *Experimental Biology and Oncology* **4** 225.
Parvathi J and Aruna K (2011a). Leucocyte Variation, an Insight of Host Defenses During Hymenolepiasis and Restoration with Praziquantel. *Indian Journal of Pharma Science* **73**(1) 76-79.
Chappell LS (1980). *Physiology of parasites.* London: Blackie.