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STUDIES OF THE MALATE DEHYDROGENASE CONTENT IN TISSUES OF *MESOCRICETUS AURATUS* INFECTED WITH *ANCYLOSTOMA CEYLANICUM*

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ABSTRACT
Tropical and subtropical areas are susceptible to helminthic infections. The endoparasitic hookworm, *Ancylostoma ceylanicum* infections damage the host's intestinal tract. Numerous laboratory animals have been observed to get hookworm infections. The anterior end of the hookworm contains a unique feature that resembles a hook and aids in the parasite's ability to take nutrients from the host. Hookworm infections result in serious abnormalities in tissue structure and function causing metabolic problems. In the current study, a hamster, *Mesocricetus auratus* served as the experimental host for the hookworm *Ancylostoma ceylanicum* infection. In the present study, malate dehydrogenase activity had been investigated in the various issues and serum of infected and non-infected golden hamster, *Mesocricetus auratus.* Increased Malate dehydrogenase content was observed in the intestine, kidney, lung and serum. Decreased MDH content was found in the liver, spleen, brain and muscle. The results of the present study suggest the significant role of MDH in the metabolism of golden hamsters infected with *Ancylostoma ceylanicum*.

***Keywords:** Ancylostoma ceylanicum, Hamster, Mesocricetus auratus, Infection, Hookworm*

INTRODUCTION
Ancylostoma(Hook Worm) causes ancylostomiasis in animals and humans. Some of the clinical symptoms include intestinal malabsorption of nutrients (Sheehy *et al.*, 1962; Tandon *et al.*, 1969), hypoalbuminemia (Roche and Layrisse, 1966), and significant intestinal blood loss resulting in iron deficiency anaemia. In golden hamsters (*Mesocricetus auratus*), the hookworm parasite *Ancylostoma ceylanicum* of cats, dogs, and humans has been effectively maintained. Visen *et al.*, (1984) worked on the model system to examine the extent of the pathogenesis of the disease. Malate dehydrogenase activity was reported from various host and parasitic animals by Karlsson and Larsson (1971), Michejda and Beczon (1971, 1972) to demonstrate and diagnose tissue parasitism.
Malate Dehydrogenase (MDH) is one of the key enzymes of the tricarboxylic acid cycle. This enzyme oxidises L-, malic acid to a glucose molecule by the sequence of glycolytic reactions, and gets converted to phosphoenol pyruvate which is transformed to oxaloacetate. It is a precursor for the whole chain reaction of the tricarboxylic acid cycle. It is linked to pyridine nucleotide and transfers hydrogen to NAD which is used as an electron acceptor (Bueding and Saz, 1968). MDH is found in all eukaryotic cells. It occurs in two forms, which are mitochondrial m-MDH and soluble or cytoplasmic s-MDH according to their location (Delbruck *et al.*, 1959). It had been reported that both the cytoplasmic and mitochondrial enzymes could occur in multiple sub-forms. Based on physical properties speed and direction of migration in electrical fields are referred to as isozymes but their physiological importance remains uncertain. Cytoplasmic s-MDH is involved in the cytoplasmic site of malate shuttle which transports malate across the mitochondrial membranes. The mitochondrial enzyme m-MDH is part of the tricarboxylic acid cycle and is responsible for the conversion of malate to oxaloacetate, thus forming the other half of the malate shuttle. Langer and Smith (1971) reported 4 isoenzymes from *Ascaris lumbricoides* and 7 from *Haemonchus contortus.*

Karlsson and Larsson (1971) reported multiple molecular forms in Mongolian gerbil, rat, mouse and rabbit. Michejda and Boczon (1971,1972) reported the isoenzyme composition of malate dehydrogenase in rat tissues during experimental trichinosis. Zee and Zinkham (1975) reported isoenzymes in the human and pig tissues of various species of *Ascaris.*

MATERIALS AND METHODS

The tissues were homogenised in cold distilled water using glass beads. The homogenate was centrifuged at 4±1°c at 2,500 rpm for 15 min. The supernatant was cooled and used to determine lipase activity by the titrimetic method of Cherry and Grandall (1932). The control and test samples were prepared. The control sample was prepared by taking 3ml of distilled water and 1ml of crude enzyme extracts into a test tube. The test tube was placed in the boiling water bath for 5 min to inactivate the enzyme. To this, 0.5ml buffer solution and 2ml of live oil emulsions were added and the contents were incubated at 37 ±1°c for 24 hrs. The test sample was prepared by taking 3ml of distilled water and 1ml of crude enzyme extract. The 0.5ml of buffer and 2ml of olive oil emulsion were added. The contents were shaken well and incubated at 37±1°c for 24 hours. After incubation period of 24hrs, 3ml of 95 % ethyl alcohol was added to each of the control and test solutions to stop the reaction. 0.2 drops of 1 % phenophthalene solution was added, mixed well and contents were titrated with 0.05N sodium hydroxide till a permanent pink colour was obtained. Lipase activity in lipase units per ml of extract = ml of NaOH used for the test sample – ml of NaOH used for control.

REVIEW OF LITERATURE

A review of related literature shows the research on the Malate dehydrogenase activity in the host infected by parasites by various researchers. Malate dehydrogenase and lactate dehydrogenase were responsible for maintenance of the cytoplasmic redox state. (free NAD/NADH). The relative activity of lactate dehydrogenase and malate dehydrogenase in *Hymenolepis diminuta* depends on the factors regulating pyruvate kinase (PK) and phosphoenol pyruvate carboxy kinase (PEPCK) (Bryant. 1975) It is the product of PK and PEPCK reactions which form the substrates i.e. pyruvate and oxaloacetate for lactate dehydrogenase and malate dehydrogenase respectively (Bueding and Saz, 1968; Carter and Fairbairn, 1975; Moon *et al.,* 1977). Thus, this type of metabolic end product excreted in *Hymenolepis diminuta* depends upon the regulatory properties of PK and PEPCK (Moon *et al.,* 1977). The literature review suggests that information regarding the influence of helminth infections in the alimentary canal on the malate dehydrogenase of various tissues of the host *Mesocricetus auratus* is inadequate. When *A. ceylanicum* was experimentally injected into hamsters, Khan *et al.,* (1988) examined the blood/serum parameters of the animals. They noticed changes in the quantities of cellular components and the release of enzymes into the bloodstream. Quinnell (1988) investigated the host age and the growth and fecundity of *Hymenolepic diminuta* in the rat. Mukherjee *et al.,* (1988) studied the biochemical alterations in golden hamsters during *Ancylostoma ceylanicum* infection. He found a decreased Malate Dehydrogenase activity in the jejunum and liver of the host infected with *Ancylostoma ceylanicum*. Studies on the infection of *Hymenolepis diminuta* with rat's intestinal helminth showed effects on exploratory behaviour and cognitive processes (Wojnar, 2022). In the present study, the author has attempted to study the malate dehydrogenase content in the tissues of hamsters (*Mesocricetus auratus*) infected with hookworm (*Ancylostoma ceylanicum*).

RESULTS

The malate dehydrogenase activity was estimated in different tissues and serum of hamsters infected with hookworm and in uninfected control. The results are given in the table no.1. The results obtained in the various tissues of the control animals are indicated as liver 3.741 ± 0.094, intestine 2.280 ± 0.266, muscle 3.288 ± 0.087, kidney 2.280 ± 0.266, spleen 1.564 ± 0.140, lung 3.114 ± 0.113 , brain 2.289 ± 0.322 µ moles of formazan / mg protein / hr and in serum 0.479 ± 0.061 µ moles of formazan / mI /hr. The values in the different tissues of the infected host as indicated in liver 1.850 ± 0.098, intestine 3.562 ± 0.053, muscle 2.666 ± 0.053, kidney 3.740 ± 0.094, spleen 0.829 ± 0.094, lung 4.211 ± 0.141, brain 1.259 ± 0.097 µ moles of formazan /mg protein /hr and in serum 0.851±0.094 µ moles of formazan/ml/hr. The malate

dehydrogenase activity was found to be increased in the intestine, kidney, lung and serum by 56.228%,59.693%, 35.228%, and 77.662% respectively. However, it was found to be decreased in the liver, muscle, spleen and brain by 50.548%, 18.917%, 46.995%, and 44.823% respectively. The above-mentioned results are statistically significant.

Table 1: Malate Dehydrogenase activity in the different tissues and serum of *Mesocricetus auratus* induced with *Ancylostoma ceylanicum* infection

S.No.	Tissues	Group	Mean ± S.D.	%Change
1.	Liver	Control	3.741 ±0.094	(50.548 %)
		Infected	1.850±0.098	
2.	Intestine	Control	2.280±0.266	(56.228 %)
		Infected	3.562±0.053	
3.	Muscle	Control	3.288 ±0.087	(18.917 %)
		Infected	2.666 ±0.053	
4.	Kidney	Control	2.280± 0.266	(59.693 %)
		Infected	3.740 ± 0.094	
5.	Spleen	Control	1,564 ± 0.140	(46.995 %)
		Infected	0.829 ± 0.094	
6.	Lung	Control	3.114± 0.113	(35.228 %)
		Infected	4.211 ± 0.141	
7.	Brain	Control	2.289 ± 0.322	(44.823 %)
		Infected	1.259± 0.097	
8.	Serum	Control	0.479 ±0.061	(77.662 %)
		Infected	0.851 ±0.094	

Table 2: 't' values calculated for different tissues and serum for Malate dehydrogenase content in *Mesocricetus auratus* induced with *Ancylostoma ceylanicum* infection

S.No.	Tissues	t-value	Probability	Remarks
1.	Liver	30.500	P<0.05	Significant
2.	Intestine	10.595	P<0.05	Significant
3.	Muscle	13.234	P<0.05	Significant
4.	Kidney	11.595	P<0.05	Significant
5.	Spleen	9.800	P<0.05	Significant
6.	Lung	13.543	P<0.05	Significant
7.	Brain	6.833	P<0.05	Significant
8.	Serum	7.294	P<0.05	Significant

DISCUSSION

Malate dehydrogenase (MDH) is one of the important enzymes of tricarboxylic acid cycle found in large quantities and responsible for the interconversions of malate-oxaloacetate. Malate never appears as an end product; for it enters the mitochondria, where it is subjected to redox dismutation. The enzyme malate dehydrogenase brings about the conversion of malate to oxaloacetate with the reduction of NAD+ in the normal tricarboxylic acid cycle. At neutral pH the equilibrium lies very much to the left. The reduction of oxaloacetate to malate is a very rapid reaction and oxaloacetate added to tissues disappears in a few seconds. Although, oxaloacetate is required for the constant operation of the citric acid cycle and is also a potent inhibitor of succinic dehydrogenase and thus any accumulation of oxaloacetate would act as an automatic cut out, preventing its formation through the operation of the cycle. In the present study quantitative estimation of Malate Dehydrogenase is carried out in golden hamsters induced with *Ancylostoma ceylanicum* infection and was found to be increased intestine, kidney, lung and serum. Mukherjee *et al.,* (1988) found decreased activity of Malate dehydrogenase in the liver of golden hamsters infected with *Ancylostoma ceylanicum* infection. The results of the present study showed reduced activity of MDH in the liver of the host infected with *Ancylostoma ceylanicum*. In addition, decreased activity in the muscle, spleen and brain suggests the metabolic synthesis of the MDH in these tissues. The interpretation of the results helps to understand the role of malate dehydrogenase in the metabolism of hamsters infected by *Ancylostoma ceylanicum*. Several changes occur in the host causing physiological alterations in certain tissues. Gastrointestinal helminth infection inflicts structural, functional and pathological changes in the various tissues of golden hamsters (*Mesocricetus auratus*). Many researchers have investigated the pathogenicity in the hamsters under helminth infection. Bannon and Friedell(1966); Tuetz(1976);Schmidt *et al.,* (1983); Maxwell *et al.,* (1985); Khan et al(1988); Srivastava *et al.,* (1988); Mukerjee *et al.,* (1988;1992).

CONCLUSION

Endoparasitic helminths of vertebrates host especially those living in the alimentary canal affect the host directly by absorbing the readily available digested food and injuring the alimentary canal's wall. *Ancylostoma ceylanicum*, a hookworm causing ancylostomiasis has a significant role in the work related to the pathophysiology of the tissues of golden hamster. As the host is deprived of its digested food, the absorption of other nutrient molecules is interfered with due to the presence of the parasites. The role of malate dehydrogenase in parasite metabolism is studied in the present investigation revealing its relevance to the host's metabolism during the parasitic infection. The work carried out conveys the regulation of the enzyme, malate dehydrogenase in parasitic adaptation.

REFERENCES

Bannon PD and Friedell GH (1966). Values for plasma constituents in normal and tumour bearing golden hamsters, *Laboratory Animal Care*, **16** 417-420.

Blecharz Klin K, Świerczyńska M, Piechal A, Wawer A, Joniec Maciejak I, Pyrzanowska J and Bryant C (1975). Carbondioxide utilization and the regulation of respiratory metabolic pathways in parasitic helminths. *Advances in Parasitology*, 13, London, USA, Academic Press.

Bueding E and Saz HJ (1968). Pyruvate kinase and phosphoenol pyruvate carboxykinase activities of Ascaris muscle, *Hymenolepis diminuta* and *Schistosoma mansoni*. *Comparative Biochemistry and Physioiologyl*, **24** 511-518.

Carter CE and Fairbairn D (1975). Multienzyme nature of pyruvate kinase during development at *Hymenolepis diminuta*(Cestoda). *Journal of Experimental Zoology*, **194** 439-448.

Cherry and Grandall (1932). In Hawk's physiological chemistry (edited by oser, B.L.,1954) McGraw-Hill book company, New York.

Delbruck A, Schmassek H, Bartsch K and Bucher T (1959). In "The enzyme XI (1975), Ed. P.D. Boyer, Academic Press, New York.

Karlsson BW and Larsson GB (1971). Lactic and malic dehydrogenases and their multiple molecular forms in the Mongolian gerbil as compared with the rat, mouse and rabbit, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistr*y, **40** (1) 93-108, https://doi.org/10.1016/0305-0491(71)90065-4.

Khan MM, Srivastava AK, Katiyar JC, Mishra A and Ghatak S (1988). *Ancylostoma ceylanicum*: Studies on host metabolism using golden hamsters as experimental model. *Rivista di parassitologia*, **549**(1): 112-117.

Langer BW and Smith, WJ (1971). The Lactic acid dehydrogenase of some parasitic gastro intestinal nematodes, *Ascaris suum, Oesophagostomum radiatum* and *Haemonchus contortus*, Comp*arative Biochemistry and Physiology*, **40B**,833-840.

Maxwell KO, Wish C, Murphy JC and Fox JG (1985). Serum chemistry reference values in two strains of Syrian hamsters, *Laboratory of Animal Science*, 35 67-70.

Michejda JW and Boczon K (1971,72). Activity and isozymic composition of lactate and malate dehydrogenases in rat tissues during experimental trichinosis. Bulletin de la societe des Amis des sciences at des letters de poznan, D. (Sciences Biologiques). **12**/13 19 - 115.

Moon TW, Herbert WC, Mustafa T and Mettrick DR (1977). A study of lactate dehydrogenase and malate dehydrogenase in adult *Hymenolepis diminuta(*cestode), *Comparative Biochemistry and. Physiology*, **56**(8) 249-254.

Mukherjee S, Tekwani BL, Tripathi LM, Maitra SC, Visen PKS, Katiyar JC and Ghatak S (1988). Biochemical and Histopathological Alterations in Golden Hamster during Infection with *Ancylostoma ceylanicum'. Experimental and Molecular Pathology*, **49**, 50-61.

Mukerjee S, Upreti RK, Tekwani BL and Kidwai AM (1992). Biochemical analysis of jejunal brush border membrane of golden hamsters, Pathogenic modulations due to ancylostomiasis, *Indian Journal of Biochemistry and. Biophysics*, **29**(1) 82-86.

Quinnell, RJ (1988). Host age and the growth and fecundity of *Hymenolepis diminuta* in the rat. *Journal of Helminthology*, **62**(2). 158 - 162. ISSN 0022-149X.

Roche M and Layrisse M (1966). The nature and causes of hookworm anaemia. *American Journal Tropical Medical Hygiene*, **15**, 1032-1100.

Schmidit RE, Eason RL, Hubbard GB, Young JT and Eisenbrandt DL (1983). Pathology of Ageing hamsters, CRC press, Boca Raton, Florida.

Srivastava JK, Suman Gupta and Katiyar JC (**1988).** Effects of CDRI compounds on energy metabolism of *Ancylostoma ceylanicu*m and *Nippostrongylus brasiliensis*, *Indian Journal of Experimental Biology*, **27**(8) 735-738.

Sheehy TW, Meroney WH, Cox RS, JR and Soler JE (1962). Hookworm disease and malabsorption, *Gastroenterology*, **42**, 148-156.

Tandon BN, Kohli, RK, Saraya AK, Ramchandran K, and Prakash O (1969). Role of parasites in the pathogenesis of intestinal malabsorption in hookworm disease. *Gut*, **10**, 293-298.

Tietz NW (1976). Fundamentals of Clinical Chemistry. Saunders Philadelphia, Pennsylvania.

Visen PKS, Katiyar JC and Sen AB (1984). Studies on infectivity, longivity and fecundity of *Ancylostoma ceylanicum* in golden hamsters, *Journal of. Helminthology*, **58**, 159-163.

Wojnar E, Zawistowska Deniziak A, Sulima Celińska A, Młocicki D, Mirowska Guzel D (2022). Infection with intestinal helminth (*Hymenolepis diminuta*) impacts exploratory behavior and cognitive processes in rats by changing the central level of neurotransmitters, *PLoS Pathogens*, 14 18(3) e1010330. doi: 10.1371/journal.ppat.1010330.

Zee DS and Zinkham WH (1975). Demonstration of parasitism in tissues by electrophoretic definition of parasite enzymes in host tissues, *Journal of Pediatrics*, **86**(3) 408 – 410.

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