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Studies of the Lipase Content in Tissues of *Mesocricetus Auratus* Infected with *Ancylostoma Ceylanicum*

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Abstract: ***Endoparasitic hookworm infections, which are typical of an intestinal host region, are the helminthic infection that affects tropical and subtropical regions. Numerous lab animals have been observed to have hookworm infections. The anterior end of the hookworm contains a unique feature that resemble a hook and aids in the parasite's ability to take nutrients from the host. Hookworm infection results in serious abnormalities in tissue structure and function as well as metabolic problems. In the current study, a hamster Mesocricetus auratus served as the experimental host for the hookworm Ancylostoma ceylanicum infection. The lipase content in different tissues of the infected subject was measured. Lactase content in both infected and control samples in M. auratus were examined. The lipase activity was found to be more in the lung tissue of the infected host. Decreased activity of lipase was found in the liver, intestine, muscle, kidney, spleen, brain and serum of the infected host. In the present study, the lipase activity in many tissues of the infected host, Mesocricetus auratus was less. The results in various tissues gave an insight into parasitic stress affecting the metabolism of Mesocricetus auratus.***
Keywords: Ancylostoma ceylanicum, Hamster, Mesocricetus auratus, Lactase, infection, hookworm.

I. INTRODUCTION

Lipases are enzymes which hydrolyse glycerol esters (Pearse, 1968). Triglycerols are significant energy stores in living organisms. Their hydrolysis releases free fatty acids whose subsequent oxidation yields about twice as much energy as the oxidation of similar weights of carbohydrate or protein. Triglycerols are stored as globules in adipocytes, forming a concentrated energy store. The first stage in preparing the stored fats for oxidation is their hydrolysis by lipase. The action of lipases is under hormonal control. The hydrolysis of triglyceride by most lipases is known to go beyond the diglyceride stage and to form a substantial amount of monoglyceride and sometimes free glycerol. The difference between esterase and lipases appear to be that the lipases are unable to attack substrate molecule fully dispersed in water. The minimum degree of molecular aggregation of the substrate compatible with lipase action is still unknown. Lipases have been reported to be present in several tissue organs of mammals like the heart, brain, muscle, adipose tissue, kidney and serum. They have been identified in milk, fish, invertebrates and also in plants and micro-organisms. Three groups of lipase enzymes have been distinguished: Lipases discharged into the digestive tract by specialised organs, tissue lipases and Milk lipases. The presence of gastic and intestinal lipases is now definitely established. In mammals, the rate of fatty acid breakdown is controlled by the rate of supply of free fatty acids. The hydrolysis of triacylglycerols is catalyzed by triacylglycerol lipase, which exists in two interconvertible forms: an active phosphorylated state and an inactive dephosphorylated state. Since most lipids are insoluble in water, the transport of these compounds in blood is accomplished by associating them with specific proteins which presumably have hydrophobic areas on their surfaces. to which lipids can bind. Triacylglycerols, phospholipids and cholesterol are transported in body fluids as particles called lipoproteins. While most lipoproteins are synthesised from endogenous fatty materials, chylomicrons are derived from dietary fat. Dietary triacylglycerols are emulsified by bile salts, and hydrolysed by digestive lipases to give fatty acids and 2-monoacylglycerols. These are absorbed by intestinal mucosal cells and used by the cell to give triacyl glycerols. The triacylglycerols are combined with cholesterol, phospholipids and proteins to form chylomicrons which are extruded from the cell into the lymphatic system, eventually reaching the blood system via the thoracic duct. Chylomicrons are removed from the circulation by the liver. Here, the triglycerols are hydrolysed to glycerol and fatty acids. The fatty acids may be oxidized to provide metabolic energy but, more importantly, are used in synthesis of other triacylglycerols for lipoprotein formation. Lipases, occur in the intestine of several nematodes *Ascaris lumbricoides*. *Ancylostoma* causes ancylostomiasis or hookworm infection. The present study aims to understand the role of the lipases in the metabolism of the infected host as studied in various tissues biochemically.

II. REVIEW OF LITERATURE

The review of literature shows the research on the Lipase activity in the host infected by parasites by various researchers. Some of the prominent research has been analysed. Tissue lipases, involved in triacylglycerol mobilisation, have been reported in helminths. Lipases was found in the intestinal region of few nematodes (Rogers,1941: Carpenter, 1952: Lee 1958). 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 anemia. In golden hamsters (*Mesocricetus auratus*), the hookworm parasite *Ancylostoma ceylanicum* of cats, dogs, and humans has been effectively maintained. This model system was investigated to study the pathogenesis of ancylostomiasis (Visen et al., 1984). Gross pathological progression is found in hookworm infection. The human sera was significant in certain pathogenic conditions. Hence, it is important from the clinical point of view to assess the functional damage to the tissue under study. The literature review suggests that information regarding the influence of helminth infections in the alimentary canal on the Lactase content of various tissues of the host *Mesocricetus auratus* is inadequate. Khan et al. (1988) examined the blood/serum parameters of the animals and examined the release of enzymes into the bloodstream, when *A. ceylanicum* was experimentally injected into hamsters. 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. Studies on the infection of *Hymenolepis diminuta* with rat's intestinal helminth showed effects on exploratory behaviour and cognitive processes (Blecharz-Klin, 2022).

III. METHODOLOGY

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 titrimetric method of Cherry and Grandall, (1932). Control and test samples were prepared as follows. The Control was prepared by adding 3ml of distilled water and 1 ml of crude enzyme extracts into a test tube. The tube was placed in the boiling water bath for 5min to inactivate the enzyme. To this 0.5 ml buffer solution and 2 ml of olive oil emulsion were added and the contents were incubated at 37 ± 1°c for 24 hrs. The test is prepared by adding 3ml of distilled water and 1ml of crude enzyme extract. To this 0.5ml of buffer and 2ml of olive oil emulsion were added. The contents were shaken and incubated at 37±1^{O}c for 24 hrs. 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. The lipase activity in lipase units per ml of extract = ml of NaOH used for the test sample used for control.ples – ml of NaOH used for control.

IV. RESULTS

The lipase activity was estimated in various tissues and serum of hamster infected with a hookworm and in uninfected control. The results are given in the table no.1, 2. The results obtained in the various tissues of the control animals are indicated as liver 78.773 ± 1.539, intestine 51.072 ± 2.195, muscle 59.136 ± 1.097, kidney 51.518 ± 2.001, spleen 59.168 ± 1.706, lung 43.456 ± 2.688, brain 55.548 ± 1.681 units / ml of extract and in serum 52.416 ± 1.792 units / ml of serum. The values in the different tissues of the infected host as indicated in liver 53.760 ± 2.004, intestine 50.176 ± 1.097, muscle 45.696 ± 3.353. kidney 47.936 ± 1.792, spleen 49.280 ± 2.405, lung 56.538 ± 1.536, brain 43.904 ± 3.038 units / ml of extract and in serum 44.800 ± 4.007 units / ml of serum. The lipase activity enhanced in lung tissue by 30.104% However, it was decreased in liver, intestine. muscle, kidney, spleen, brain, and serum by 31.753%, 1.754%, 22.727%, 6.953%, 13.712%, 20.962%, and 14.530% respectively

Table 1

Percent of lipase content 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	78.773±1.539	(31.753%)
		Infected	53.760±2.004	
2.	Intestine	Control	51.072±2.195	(1.754%)
		Infected	50.176±1.097	
3.	Muscle	Control	59.136±1.097	(22.727%)
		Infected	45.696 ±3.353	
4.	Kidney	Control	51.518± 2.001	(6.953%)

		Infected	47.936 ±1.792	
5.	Spleen	Control	59.168± 1.706	(16.712 %)
		Infected	49.280 ±2.405	
6.	Lung	Control	43.456 ±2.688	(30.104 %)
		Infected	56.538 ±1.536	
7.	Brain	Control	55.548± 1.681	(20.962 %)
		Infected	43.904± 3.038	
8.	Serum	Control	52.416± 1.792	(14.530 %)
		Infected	44.800 ±4.007	

For tissues, values are expressed as units/ml of extract
For serum, values are expressed as µ glucose/ml of serum
± indicates the standard deviation for control and experimental
Figures in parenthesis is percent change over control.

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

S.No.	Tissues	t-value	Probability	Remarks
1.	Liver	22.155	P<0.05	Significant
2.	Intestine	0.181	P<0.05	Insignificant
3.	Muscle	32.861	P<0.05	Significant
4.	Kidney	3.512	P<0.05	Significant
5.	Spleen	1.413	P<0.05	Insignificant
6.	Lung	14.832*	P<0.05	Significant
7.	Brain	7.503	P<0.05	Significant
8.	Serum	4.034	P<0.05	Significant

*Indicates absolute value of 't'

V. DISCUSSION

The deposition of fats in the tissue is controlled by the clearing factor lipase, which in a state of calorie excess is high in capillaries of the adipose tissue. Thus, blood triglycerides are hydrolysed and fatty acid enters the adipose tissue to be stored as triglyceride. If the calorie excess is from carbohydrate, the triglycerides in the blood are largely those manufactured by the liver from the carbohydrate. If the calorie excess is from dietary fat the triglycerides will be largely from the intestine in the form of chylomicrons. Clearing factor lipase is also found at high activity in calorie excess in the skeletal and heart muscles, in the kidney and the lung. In times of calorie deficiency where the blood triglycerides originate from the liver (using fatty acids supplied from the adipose tissue) the clearing factor lipase of the adipose tissue falls to very low levels where as the activity in the muscle increases, thus positively directing the fatty acids towards the muscle for oxidation. Increased level of lipase activity in the lung tissues of the infected host indicates that it continues lipolysis and reesterification of the stored triacylglycerols takes place resulting in the formation of free fatty acids and glycerols. Since glycerol cannot be utilized readily by the tissues, it diffuses into the plasma and becomes active when it is utilised by these tissues. The free fatty acids in the tissues form acyl CoA which may either partially enter the reesterification cycle or the remaining part may enter into the tricarboxylic acid cycle to compensate for the high energy demands of the infected host. The enzyme which makes the triglyceride store of the adipose tissue available is the hormone-sensitive lipase. The activity of this enzyme determines the level of free fatty acids (attached to albumin) in the blood and also the level of circulating glycerol. There is no special mechanism available to tap the stores of proteins but it is known that all cells contain proteinases and all cells have a pool of free amino acids. The pool of amino acids and the cellular proteins are constantly turning over and blood aminoacids are constantly entering the cellular pool. It may be that in times of blood amino acids, these compounds are naturally moved from the cellular pool into the blood.

Mukerjee et al,(1992) carried out work related to the infection of *Ancylostoma ceylanicum* in golden hamsters by the model system. Significant biochemical modulations were observed in the hamster jejunal brush border membrane (BBM), the primary site of infection. Analysis of BBM at the peak of infection (3 weeks) revealed a marked decrease in the activities of lactase. These results reveal that hookworm infection causes severe damage not only to the site of attachment alone but also to the entire cell lining of the jejunum and therefore could influence overall digestion and absorption.

In the present study quantitative estimation of lipase activity was carried out in golden hamsters induced with *Ancylostoma ceylanicum* infection. The decreased lipase activity level in the infected host tissues of the liver, intestine, muscle, kidney, spleen, brain and serum indicates the accumulation of the total lipids(triglycerides) which inturn are utilized by the β-oxidation process to meet the high energy demands if the tissue. The increased lipase activity was observed in the lungs of the infected host. Several physiological changes occur in the host. Gastrointestinal helminth infection inflicts the structure, and function of tissues of the host. These infections caused 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);(1982); Schmidt et al.(1982); Maxwell et al(1985); Khan et al(1988); Srivastava et al(1988); Mukerjee et al.(1988).

VI. 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. The role of lipase content in parasite metabolism was studied in the present investigation revealing its relevance to the host's metabolism during the parasitic infection. The hookworm infection results in serious abnormalities in tissue structure and function. In the current study, a hamster, *Mesocricetus auratus* served as the experimental host for the hookworm *Ancylostoma ceylanicum* infection. Lipase content was reported in the infected and control tissues of the host. The results suggested that infected by *Ancylostoma ceylanicum* in *Mesocricetus auratus* caused the altered physiology of the host as seen by the difference in lipase activity in the tissues.

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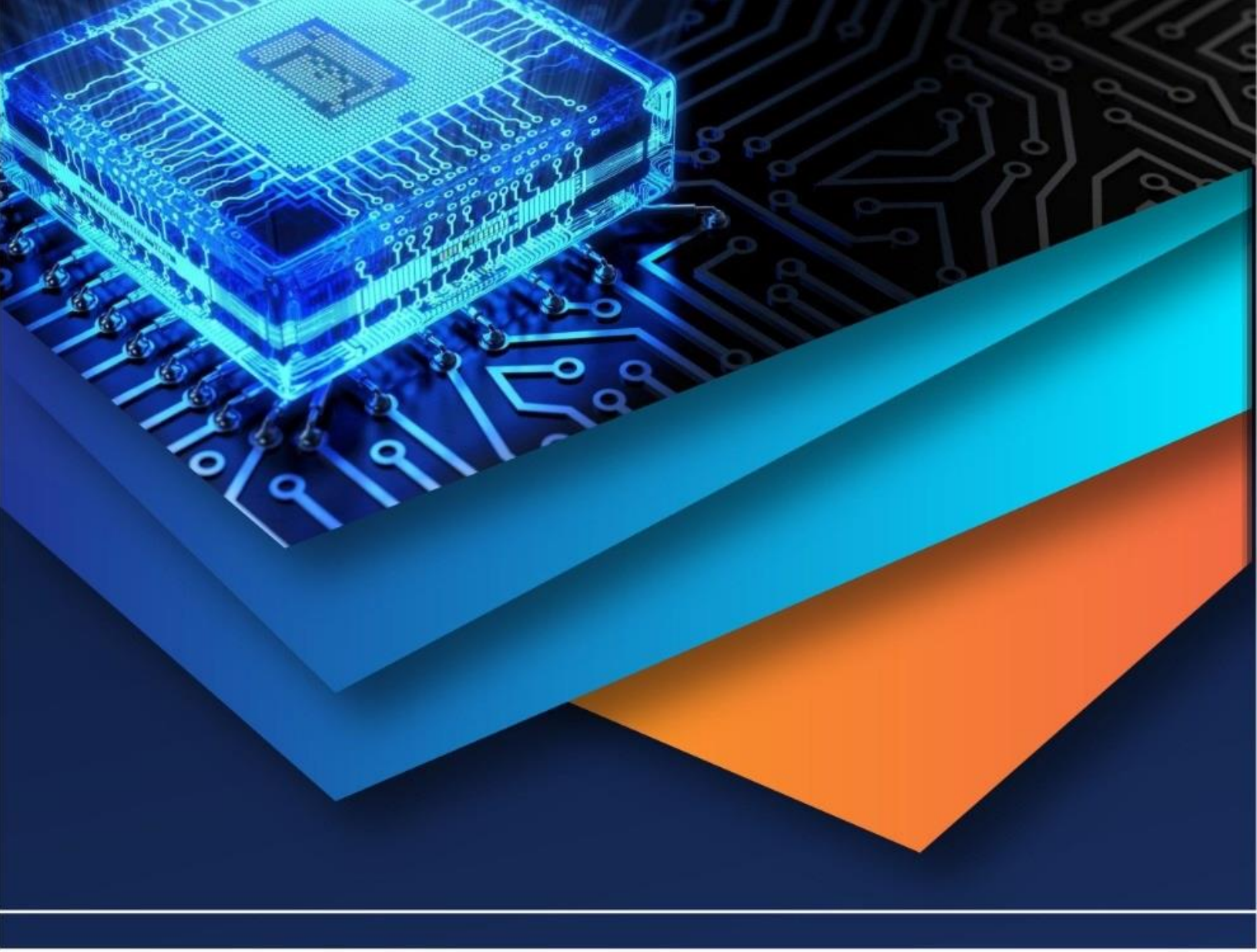

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