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STUDIES ON THE GLYCOLIPIDS CONTENT IN HAMSTERS (*MESOCRICETUS AURATUS*) INFECTED WITH *ANCYLOSTOMA CEYLANICUM*

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ABSTRACT

The helminthic infections are among the most prevalent helminthic infections, particularly in tropical and subtropical regions is an endoparasitic hookworm infection characteristic of intestinal region of the host. Hookworm infections have been reported in several lab animals. The hookworm has a specific hook-like structure at the anterior end that helps the parasite to extract nutrients from the host. Metabolic disorders and severe disruptions in tissue structure and function are caused by hookworm infection. In the current investigation, *Ancylostoma ceylanicum,* a hookworm was infected in the host, a hamster *Mesocricetus auratus* experimentally. The infected host was then studied by taking the parameters of glycolipids in various tissues. Glycolipids play an important role in lipid metabolism. The glycolipids content in *M. auratus* was investigated biochemically in both the infected and control samples. The total glycolipids content was estimated biochemically in the hamsters and results revealed that the tissues had enhanced levels. The results showed increased glycolipids content in the intestine, muscle, liver, kidney, spleen, lungs, brain and serum. The total amount of glycolipids in the host tissues, *M. auratus*, can help determine the pathogenicity level of *A. ceylanicum* infection. The present paper reveals the intricacies of the glycolipid level in the infected Hamster and its relevance in enzyme biochemistry.

INTRODUCTION

Hookworm infections are among the most prevalent helminthic infections, particularly in tropical and subtropical regions, where they contribute to significant morbidity (Pearson *et al.,*2012). The lifecycle of hookworms involves intestinal colonization, where they attach to the mucosa using specialized hook-like structures at their anterior end. This attachment facilitates nutrient extraction from the host, leading to tissue damage and metabolic disturbances. Among hookworm species, *Ancylostoma ceylanicum* is recognized for its zoonotic potential, affecting both humans and animals. Abuzeid *et al.*(2020) reviewed the

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knowledge about hookworm exceretory/secretory products, including their role in parasite biology, host-parasite interactions, and as vaccine and pharmaceutical targets identifying research gaps in future investigations. Mendez *et al.*(2005) worked the host antibody responses during the *A. ceylanicum* infection in Syrian hamsters.

Lipid metabolism plays a crucial role in maintaining cellular functions, and glycolipids are key components involved in cellular signaling, membrane integrity, and energy storage (Cornelis *et al.*,2017).

Lipids are a heterogeneous group of organic compounds occurring in all animal tissues. They are generally esters of long-chain fatty acids and important energy reserves and form a major component of the cell membrane Lipids has a common property of insolubility (relatively soluble in water),but it is soluble in non-polar organic solvents such as chloroform, alcohol, acetone, ether, and benzene. They rarely exist in simple form in an organism but are often found associated with carbohydrates and proteins as glycolipids and lipoproteins (Berriozabalgoitia *et al.*,2017).

Glycolipids are cerebrisides or glycosphingosides in general resemble the sphingomyelins in structure (Svennerholm, 2024). Glycolipids are formed by using a ceramide backbone.the two groups of glycolipids: Cerebrisides, contain a single fatty acid chain, a complex alcohol (usually sphingosine) and one or more hexose sugars(usually galactose) and gangliosides which are chemically similar to cerebrosides but also contain neuraminic acid or its derivatives (Wuhrer *et al.*, 2002). They are more complex glycosphingolipids containing more sugar residues plus sialic acid. They are present in the outer layer of the plasma membrane, where they contribute to the glycocalyx and are important as antigens and cell receptors. Glycolipids are formed by the sequential addition of sugars to the ceramide.

Glycolipids are characterised by the presence of galactose in the molecule for which they are also known as galactolipids. They contain fatty acids,galactose and nitrogenous base sphingosine. They account for 5 to 10% of the lipids of the plasma membrane in lipids. They are abundant in the white matter and myelin sheath of the brain (Makaaru *et al.*1992 & Luh *et a*l.,2024). During pathological conditions, cerebrosides have been found to occur in large amounts found in liver, spleen, brain, kidney, salivary glands and testes. Alterations in glycolipid levels are indicative of metabolic stress and pathological conditions. The current study focuses on the biochemical assessment of glycolipids in *M. auratus* following experimental infection with *A. ceylanicum*, aiming to elucidate the metabolic impact of the infection on host tissues.

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MATERIALS AND METHODS

Adult male Syrian golden hamsters,*Mesocricetus auratus* of 4-5 weeks weighing around 40-50 gms were used in all the experiments. They were infected with the larva of *Ancylostoma ceylanicum*. After infection, hamsters were maintained under similar conditions of diet and environment. On day 15 microscopic examination of faecal pellets was done for the presence of *A.ceylanicum* ova. Mostly, all the hamsters were found positive for *A. ceylanicum* infection. The strain was maintained by regular passage in hamsters once in a month.

The hamsters were anaesthetized with ether by placing them in a container. The small intestines were removed for observing theAncylostoma ceylanicum infection. The fresh tissues like liver, intestine, spleen, kidney, lungs, muscle, brain and serum were collected. The tissues were homogenized and then used for biochemical estimations of glycolipids by the method followed from" A manual of laboratory techniques" by Raghuramulu *et al.(*1983).

Procedure: Different concentrations (20-200 mg) of galactose standard solution are taken in test tube and 1 ml of 2% phenol is added followed by 4 ml of conc.H_2SO4. The orange colour appears, which is measured, after cooling to room temperature for 15 min. at 480 nm. For biological samples, hydrolysis is done with 2ml 2N H_2SO_4 for 2 hrs (the time can be varied depending upon the source of the sample). After hydrolysis is over,4ml of chloroform is added and the mixture is centrifuged. From the top aqueous layer 1ml is taken out separately. To this 50 ml of 80% phenol is added followed by 4 ml of conc. H_2SO_4. The orange colour developed from the standard curve. This is then multiplied by 4.45 to estimate the glycolipids. The values of glycolipids are expressed as mg glycolipids/g wet weight of tissue.

RESULTS

The glycolipid content was estimated in various tissues and serum of hamsters infected with hookworms and in uninfected controls. The results obtained in the various tissues of the control animals are indicated as Liver 0.767 ± 0.061, Intestine 0.482 ± 0.026, Muscle 0.332 ± 0.840, Kidney 0.327 ± 0.014, Spleen 0.510 ± 0.045, Lung 0.375 ± 0.060, Brain 0.711 ± 0.074 mg glycolipid /g wet weight of tissue and in serum 0.196 ± 0. 022 mg glycolipid 100 ml of serum.

The values in the different tissues of the infected host indicated in Liver 0.986± 0.020, Intestine 0.580 ± 0.067, Muscle 0.583 ± 0.104, Kidney 0.582± 0.090,

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Spleen 0.823 ± 0.074, Lung 0.795 ± 0.120, Brain 0.991 ± 0.060 mg glycolipid 7g wet weight of tissue and in serum 0.308 ±+ 0.021 mg glycolipid /100 ml serum. The glycolipid content has been increased in various tissues like the liver. intestine, muscle, kidney, spleen, lung, brain, and serum by 28.553%, 20.332%, 42.955%, 43.816%, 61.373%, 52.830%, 39.381% and 36.364% respectively. The results were statistically significant. The tabular representation is given in Table 1 and Histogram 1

Table 1

Glycolipid 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	0.767 ± 0.061	
		Infected	0.986 ± 0.020	28.553 %
2.	Intestine	Control	0.482 ± 0.026	
		Infected	0.580 ± 0.067	20.332 %
3.	Muscle	Control	0.332 ±0.840	
		Infected	0.583 ±0.104	42.955 %
4.	Kidney	Control	0.327 ± 0.014	
		Infected	0.582 ± 0.090	43.816 %
5.	Spleen	Control	0.510 ± 0.045	
		Infected	0.823 ± 0.074	61.373 %
6.	Lung	Control	0.375 ± 0.060	
		Infected	0.795 ± 0.120	52.830 %
7.	Brain	Control	0.711 ± 0.074	
		Infected	0.991± 0.060	39.381 %
8.	Serum	Control	0.196 ± 0.022	
		Infected	0.308 ± 0.021	36.364 %

For tissues, values are expressed as mg lipid/g wet weight of tissue.

For serum, values are expressed as mg lipid/100 ml of serum.

+ Indicates standard deviation for control and experimental

Figures in parenthesis is percent change over control.

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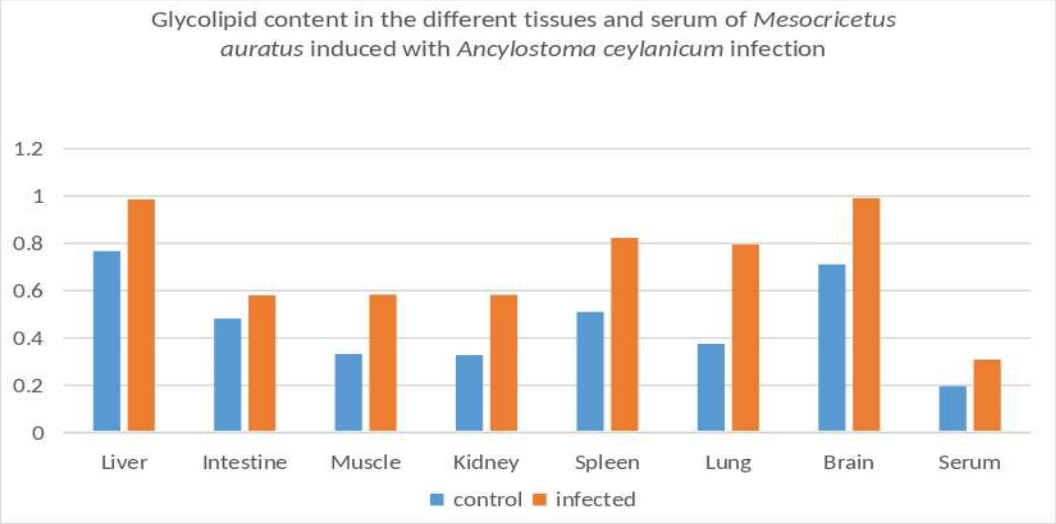
Histogram 1



DISCUSSION

Studies made in various tissues of hamsters induced with *Ancylostoma ceylanicum* infection indicated increased level of lipid content in the tissues when compared to the control. Denyes and Baumber, (1965); Mitruka and Rawnsley (1977); Thomas *et al*. (1979). Maxwell *et al*. (1985) studied lipid content levels during hibernation and starvation conditions and showed increased lipid content in the tissues of the hamsters. In the present study, increased level of total lipid content could be justified taking into account heavy worm burden in the intestine, may have caused starvation effect on the host. It is well known fact that high carbohydrate content in the diet can result in increased accumulation of body fat, thus it is evident that complete catabolism of fats depends on the carbohydrate supply (Smith and Wood, 1991). The distribution of fat varies in different tissues: for example, 50% in subcutaneous tissues, 15% in perirenal tissues, 20% mesentry, 10% omentum and 5% in the intramuscular connective tissues. (Chatterjee, 1987).

Liver:

During the period of starvation, free fatty acids arrive at the liver in large amounts than during normal conditions, since the rate of free fatty acid release from adipose tissue increases as the level of blood glucose falls. Liver to some extent, is able to compensate for this increased arrival of free fatty acids by increasing the rates of various pathways able to metabolise fatty acids. including,

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triglyceride synthesis and export. However, during even moderate starvation, there is a tendency for triglycerides to accumulate in the liver. With the large amounts of free fatty acids in the liver, despite increased metabolic activity triglycerides accumulate resulting in fatty liver (Banks *et al.* 1976) supporting the increased total lipid content observed in the liver of infected host. Increased total lipid content in the intestine has been observed. Triglycerols are a major reserve of energy mobilised when organism is energy deficient. They are synthesised principally in the liver, gut and the adipocytes of animals (Kuksis, 1978).

Intestine:

Intestinal parasites do not oxidise carbohydrates completely to carbondioxide and water, but partly oxidised energy rich metabolites are excreted in the lumen of the host (Von Brand, 1973). The absence of classical tricarboxylic acid cycle limits the further catabolism of the acetyl-CoA produced by ß-oxidation. The large amount of NADH and reduced flavoprotein formed during ß-oxidation require oxidase system. The ß-oxidation pathway cannot function anaerobically and such metabolites may be used by the host for the synthesis. Mukerjee *et al.*(1992) also reported increase in the phospholipid content in jejunal brush border or intestine of infected hamsters.

Muscle:

Increased glycolipid content was observed in the infected muscle tissue when compared to the normal. It could be due to extensive metabolic disruptions caused by hookworm infection.

Kidney:

Increased level of lipid content in the kidney was observed during parasitism indicating that during disease or starvation, there is an accumulation of ketone bodies and their subsequent excretion through urine. In a normal animal level of ketone bodies is 1mg/dl but in diseased conditions, the level may go up to 100mg/dl (Rama Krishnan *et al.,* 1994).

Spleen:

Increased serum lipid levels in the infected hamster in response to infection had been observed in the present study. Serum lipid levels and their fractions were found to be increased in the infected Gujaral *et al.* (1981) due to enhanced cholestrogenesis and lipogenesis and altered level of chylomicron from the blood. Increased serum lipid level was also reported by Denyes and Baumber, (1965); Cox and Gokcen, (1974); Maxwell *et al.* (1985) during hibernation and

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starvation. Similar findings are observed in the serum of infected hamsters. Chatterjee(1987) had reported enlargement of the spleen in certain pathological conditions resulting in the accumulation of lipids.

Lungs:

In the present investigation, high glycolipid content was reported in the infected lungs when compared to the control. It could be accounted by the fact that substantial amount of free fatty acid becomes liberated by the lung for synthesis into phospholipids. The alveoli are coated with several layers of phospholipids to reduce surface tension and as the lungs expand, the molecules slide over each other exposing the lower layers of phospholipid ensuring intact film at all lung volumes. It is therefore possible that during the respiration distress syndrome may cause the high surface tension in the alveoli due to lack of the phospholipid layer such a condition may arise during pathogenic condition evidenced by decreased level of phospholipid content in the lung during infection.

Brain:

The glycolipids found in the brain tissue infected with *A. ceylanicum* was higher compared to the control. The brain is characterised by its high content of lipid that accounts for 56% of the total dry weight of white matter and 32% of the grey matter. Most of the lipids are metabolically inert, especially those found in myelin, and a structural rather than a metabolic rate. However, there is a small fraction of phospholipids which turns over rapidly. This high rate of turnover is shared by brain phosphoproteins and is related to the level of neuronal activity. Under conditions when glucose is readily available, the flow of free fatty acids is matched by the liver's ability to oxidize them to carbon dioxide and water and to convert them to triglyceride. However, as the rate of the arrival of free fatty acids rises as happens during stress conditions (heavy work, high fat feeders, starvation), they are diverted more and more towards ketone body formation. The physiological significance is that an insoluble fuel unable to enter the brain's cells is transformed into a water-soluble material that can leave the capillaries and easily enter the cells. Ketone bodies can supply much of the brain's energy requirements during starvation stress and are oxidized in preference to glucose and fatty acids by many other tissues (Banks *et al.* 1976).

Serum:

The lipids found in the different tissues and serum are cholesterol, phospholipids, triglycerides and fatty acids. In the hamsters, circulating lipids in general are much lower than human levels (Mitruka and Rawnsley, 1977) but higher than other rodents (Cox and Gokcen, 1974).

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During deprivation of sugars as in starvation stress inadequate production of energy which may result the animal to tap fat from adipose tissue and extensive mobilisation of fat in the form of fatty acids and glycerols. Fatty acids are carried by albumin to the liver and undergo ß-oxidation, whereas glycerol is free. Both enter the liver where the glycerol is phosphorylated with a major option of being used for triglyceride synthesis or gluconeogenesis. The fatty acids have the major option of being converted to triglycerides or to ketone bodies. (Banks *et al.*, 1976).

Glycolipids in different tissues studied:

Glycolipids found in the brain, liver, intestine, muscle. kidney, lungs, spleen and serum have found to be increased during the pathogenic condition, in the present study. The observed increase in glycolipid content across multiple tissues underscores the metabolic impact of *A. ceylanicum* infection. Glycolipids, being integral to cell membranes and signalling pathways, are likely regulated as a host response to tissue damage and parasitic nutrient extraction. The intestine, being the primary site of infection, exhibited the higher glycolipid alterations, consistent with localized damage and inflammation. The systemic increase in glycolipid levels in other tissues, including the liver and kidneys, indicates broader metabolic implications of hookworm infection. These findings align with previous reports highlighting the metabolic burden imposed by parasitic infections. The observed glycolipid dynamics could serve as potential biomarkers for assessing the severity and progression of hookworm infections. Similar findings have been reported by Chatterjee(1987) during Gaucher's disease. Tay-sacchs disease observed increased cerebrosides and lipid accumulation.

Animals can synthesize phospholipids under suitable conditions. Liver can synthesize phospholipids from the intermediate product of fat oxidation. They are also synthesized in the intestinal epithelium during the absorption of fat. All tissues contain a constant amount of lipids with a characteristic composition that indicates that the cells of different tissues to some extent synthesise their own phospholipids. Brain, liver, heart and muscle being particularly rich in phospholipids. Phospholipids are involved in several disease processes including respiratory distress syndrome (lack of lung surfactant), multiple sclerosis (demyelination) and sphingolipidoses (inability to breakdown sphingolipids in lysosomes due to inherited defects in hydrolase enzymes). The systemic increase in glycolipid levels in other tissues, including the liver and kidneys, indicates broader metabolic implications of hookworm infection. These findings align with previous reports highlighting the metabolic burden imposed by parasitic

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infections. The observed glycolipid dynamics could serve as potential biomarkers for assessing the severity and progression of hookworm infections.

CONCLUSION

Glycolipids play an important role in lipid metabolism. In the present study, *Ancylostoma ceylanicum,* infection in the host *Mesocricetus auratus* experimentally provided insights into the biochemical effects of *A. ceylanicum* on glycolipid metabolism in *M. auratus*. The significant alterations in glycolipid levels across various tissues highlight the extensive metabolic disruptions caused by hookworm infections. These findings have implications for understanding the pathophysiology of helminthic infections and developing targeted therapeutic interventions.

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