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Effect of parasitic infection on rate of respiration of the freshwater pulmonate vector snail *Lymnaea acuminata* during patency period from Aurangabad city, Maharashtra, India.

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Abstract

There is paucity of information regarding the metabolic rates of trematode larval pathogens and their associated intermediate snails host in the field of pathobiology. The present investigation prompted to undertake to find out impact of larval trematode parasitic infection on rate of oxygen consumption in the intermediate snail host *Lymnaea acuminata*, a very common inhabitant-pulmonate gastropod mollusc in various types of water bodies in and around the city, Aurangabad. The present topic deals with the rate of oxygen consumption in infected and non-infected snails of *L. acuminata* during period of patency which lasts for 7 days in this snail. In initial phase of patency there is increasing rate of oxygen consumption but as snails reaches to last phase there is gradually decrease in it.

Key words- infection, respiration, vector snail, *L. acuminata*, patency

Introduction

The respiratory potential of animal is an important parameter to assess the type of stress as it is valuable indictor of energy expenditure and metabolism in general. According to the opinions of some workers in the field of respiratory metabolism of molluscs it is the fact that the oxygen consumption rate of infected snails is not reduced compared to that of non-infected snail come as no surprise because it was found by Sturrock (1966) for *Biomphalaria pfeifferi* and Thornhill *et al.*, (1986) for *B. glabrata* that *Schistosoma mansoni*, infected at all ages of the snail caused temporary acceleration of the growth rate whilst the life span was considerably shortened.

In many species, a large mass of metabolically active cercariae develop in advance infections. These may compete for oxygen with their hosts and could also impair oxygen uptake through direct pressure on the bronchial or by indirect means, such as causing anaemia; (Moutsen and Jensen 1997). Evidence in support of this suggestion comes from Sousa and Gleason (1989) who found that exposure to low level of oxygen cause differentially high mortality in infected marsh snails (*Cerithidia californica*). Tallmark and Norrgren (1976) who reported increased oxygen consumption in infected *Nassarius reticulatus*. Increased O_2 demand and reduction in the efficiency of O_2 uptake, have also been suggested as the cause of ecologically important behavioural changes, such as infected host crawling on the surface of the mud, where ambient O_2 level are highest and where they are susceptible to predation (Swennen 1969, Mourtisen and Jensen, 1997). The metabolic response of an organism to change in environmental conditions is an overall indicator of adaptive capacity of an organism. Generally the metabolic status of an organism is assessed by measuring rate of oxygen consumption. The rate of oxygen consumption by animal as a whole and its tissues as specific indicates its respective metabolic rates.

Since the physiological processes of mollusc are correlated primarily with fluctuation within the environment and molluscs are more diverse in form and physiology than any other invertebrate phyla. There is paucity of information regarding the metabolic rates of trematode larval pathogens and their associated intermediate snails host in the field of pathobiology. Hence prompted to undertake the present investigation to find out impact of larval trematode parasitic infection on rate of oxygen consumption in the intermediate snail host *Lymnaea acuminata*. The present topic deals with the rate of oxygen consumption in infected and non-infected snails of *L. acuminata* during start of cercarial release period to completion of cercarial release period i.e. during period of patency which lasts for 7 days in this snail.

MATERIAL AND METHODS

Collection of freshwater snail, *Lymnaea acuminata* was done from the different water bodies such as Salim Ali Lake, Kham River and other water bodies around the city Aurangabad during different seasons of the year 2009 and 2010. After bringing those snails to the laboratory, washed with tap water in order to remove mud and algal material attached to the shell. The snails were maintained in the laboratory in dechlorinated tap water with algal material and aquatic plants *ad libitum* in the water troughs. During parasitic infection period i.e. in the late rainy season or early winter majority of the snails of the snails species found infected with larval trematode pathogens. Naturally infected snails got sorted out and maintained separately from non-infected snails. The total cercarial release period during which there is spontaneous release of cercariae, lasts for 7 days and depending upon number of cercarial released per day this patency period can be divided into three different phases.

a) Initial phase of patency (1st and 2nd day)
b) Peak period of patency (3rd, 4th and 5th day)
c) Last phase of patency (6th and 7th day)

Oxygen consumption experiment was set with animals showing start of release of cercaria and a batch of non-infected snails also maintained simultaneously as control to check and compare the rate of oxygen consumption in infected and non-infected snails. During each phase, after every 4 hrs. of interval, rate of O_2 consumption by *L. acuminata* was observed for 24 hrs. time period. Three to four normal sized (20±2 mm shell length) snails showing foot movements from both stocks i. e. infected and non-infected were selected and transferred singly to air tight respiratory jars of 100 ml capacity as experimental (infected) and control (non-infected) snails during initial phase of patency. The rate of oxygen consumption was checked at 8.00 a.m., 12.00 noon, 16.00 p.m., 20.00p.m., 24.00 p.m. and 4.00 a.m. After one hr. consumption by both group of snails, amount of oxygen present in experimental and control snail water was calculated with the help of Standard Winkler's Technique (Welsh and Smith 1961). Before tabulation of the results an average values of oxygen consumed by three different snails from both infected and non-infected groups was calculated along with Standard Deviation. Likewise the amount of oxygen consumed or rate of oxygen consumption by *L. acuminata* was studied for another two phases of patency i.e. peak and last-phase of patency at different time intervals of the day mentioned earlier. The oxygen consumption data subjected for statistical analysis to calculate an average and standard deviation (Finney, 1964)

OBSERVATION AND RESULT

The amount of oxygen consumed by normal and infected snail *Lymnaea acuminata* during initial phase of patency was calculated and depicted in the form of observation table. From the table, it is evident that there is a slight change in rhythmicity in rate of oxygen consumption. In the morning at 8.00 a.m. there is an increase in the O_2 consumption (0.0642±0.0011 mg/liter/hr./gm. body weight) and least amount of O_2 consumed by the control snails at 20.00p.m. (0.0632±0.0017 mg/liter/hr./gm. body wt.) But compared this data with infected snails, during initial phase of patency, there is overall an increase in the rate of oxygen consumption. Maximum amount of O_2 was consumed by infected snails at 20.00 p.m. (0.0784±0.0034), while it was minimum at 4.00 a.m. (0.0755±0.0042). But these values are more, when compared with the values of O_2 consumption by normal non-infected snails. During peak phase of patency there is an intense release of cercariae. Since there is an increase in the development of cercaria by vegetative growth, may be due to this fact, there is significant increase in the rate of oxygen consumption as compare to same infected snails during initial phase of patency. Maximum amount of O_2 was consumed by infected snail at 20.00 p.m. (0.0972±0.0009) while minimum at 4 a.m. (0.0910±0.0078) during 24 hrs. of observation period after every 4 hrs. of time interval during peak phase of patency. Non- infected control snail have the more or less steady rate of O_2 consumption.

On the sixth and seventh day of patency period i.e. in last-phase of patency during which there is decreased number of cercarial release observe. During last-phase of patency, there is decreased rate of oxygen consumption by infected snails compared with both earlier phases of patency. During 24 hours of observation, at 8.00 a.m. maximum amount of oxygen was consumed (0.0751±0.0063) and minimum amount of oxygen was consumed at 4.00 a.m. (0.0700±0.0093). Though there is continuous decrease in the rate of oxygen consumed by the infected snails during last-phase of patency but compared with that of normal non-parasitized snails, infected one having more O_2 consumption rate. If comparison is made with that of the infected snails at initial phase of patency, the amount of oxygen consumed by infected snails in last phase of patency is less, when observations made for 24 hours of post patency period. The overall range of oxygen consumption during entire period of patency by non-infected snails ranges from 0.0630±0.0016 as minimum at 16.00 p.m. to 0.0672±0.0042 as maximum at 8.00 a.m. again for infected snail from 0.0700±0.0093 as minimum at 4.00 a.m. to 0.0972±0.0009 as maximum at 20.00 p.m.

Snail category	Amount of Oxygen consume in mg O_2/liter/hr/gm. Body weight at different hours during initial phase of the day					
Time	**8:00 a.m.**	**12:00 noon**	**16:00 p.m.**	**20:00p.m.**	**24:00 mid night**	**4:00 a.m.**
Non-infected	0.0642± 0.0011	0.0638± 0.0020	0.0630± 0.0016	0.0632± 0.0017	0.0635± 0.0014	0.0640± 0.0013
Infected	0.0781± 0.0036	0.0778± 0.0021	0.00780± 0.0022	0.00784± 0.0034	0.00779± 0.0010	0.00755± 0.0042
	Amount of Oxygen consume in mg O_2/liter/hr/gm. Body weight at different hours during peak phase of the day					
Non-infected	0.0655± 0.0051	0.0650± 0.0090	0.0648± 0.0068	0.0635± 0.0012	0.0631± 0.0023	0.0632± 0.0036
Infected	0.0942± 0.0063	0.0944± 0.0032	0.0948± 0.0049	0.0972± 0.0009	0.0970± 0.0067	0.0910± 0.0078
	Amount of Oxygen consume in mg O_2/liter/hr/gm. Body weight at different hours during last phase of the day					
Non-infected	0.0672± 0.0042	0.0651± 0.0013	0.0641± 0.0071	0.0642± 0.0053	0.0640± 0.0080	0.0668± 0.0066
Infected	0.0751± 0.0063	0.0745± 0.0078	0.0740± 0.0055	0.0714± 0.0049	0.0704± 0.0049	0.0700± 0.0093

Table - Amount of oxygen consumption during different phases of patency of snail *Lymnaea acuminata*

DISCUSSION

The freshwater pulmonate intermediate host snail *Lymnaea acuminata* gets parasitized naturally at its habitat and exhibit remarkable change in its respiratory metabolism during the patency period. After getting infected in the nature with larval trematode pathogens, there is vegetative growth of parasites in the snail body. The snail accommodates enormous number of trematode larvae in various stages of development. Because of this fact, there is total change in various physiological processes in the body of snail host VIZ basal metabolic rate and reproduction etc. In order to check the impact of parasitic infection, on metabolism of snail, *L. acuminata* the respiratory parameter i.e. rate of oxygen consumption or any change in measurement of rate of oxygen consumption by animal is very useful.

According to Bertalanffy (1957) the relation between metabolic rate and body size for pulmonates varies in such a way that the respiration in some cases is proportional to the body surface (Brand et al. 1948) and in some cases is intermediate i.e. proportional to more than 2/3 but less than 3/3 power of weight, for instance in the species of *Lymnaea*, *Planorbis* and *Isidora* according to experiments respectively by Bertalanffy and Muller (1943), Fosser and Kroger (1951) and Krywienczyk (1952 b). Vernberg and Hunter (1963) reported an increase in respiration rate in rediae of *Himasthala quissetensis* in vitro study. The period of patency of large sized *L. acuminata* infected with trematode pathogens at their habitat having most of the matured redie in the body of snail, hence increased rate of respiration in this snail agrees in vitro study on rediae of *Himasthala quissetensis* showed an increased rate of respiration.

The present freshwater intermediate host snail, *L. acuminata* is found distributed in both type of lentic and lotic waters in and around the city Aurangabad and seems to possess a considerably greater physiological adaptability than other molluses. Comparatively small differences as to respiration of the freshwater snails examine are probably connected with their ability to live together in ecologically very different places. Nevertheless, for some of them the oxygen conditions in a certain locality may be of decisive importance.

The gastropod pulmonate snail, *L. acuminata* shows rhythmicity in uptake of oxygen during 24 hours day-night cycle, studied in the laboratory during infestation period i.e. in early winter season. Similar type of variation in oxygen consumption of *Ancylus fluviatilis* has been observed in the course of annual cycle (Berg et. al. 1958). Naturally infected *L. acuminata* with trematode larval pathogens shows increased rate of oxygen consumption compared with non- parasitized snails. According to Sturrock (1966) for *Biomphalaria pfeifferi* and Thornhill et al. (1986) for *B. glabrata* that *Schistosoma mansoni* infected snails the fact is that the oxygen consumption rate of infected snails is not reduced compared to that of non-infected snails. VO_2 (Volume of oxygen) measurements made by Lee and Cheng (1971), however showed that *Biomphalaria glabrata* parasitized by *S. mansoni* required more oxygen, but they fail to explain why more oxygen is consumed by the snail during 6^{th} to 8^{th} week of infection. In the present investigation, naturally infected snails during their cercarial flushing period i.e. right from start of period of patency to end of the patency, there is an increase in the rate of oxygen consumption with a significant increase during peak period of patency. During this period more cercaria are developed within the infected snail body, might be affected basal metabolic rate of animal thereby causing increased oxygen consumption in parasitized snails compared with non-parasitized ones which is in accordance to earlier work by Vernberg and Hunter (1963), are of the opinion that there is an increase in respiration rate of Redia of *Himasthata*. Lee and Cheng (1971) found that the oxygen consumption rate measures for different groups of infected snail shows a similar type of significant increase during the week's 6^{th} and 7^{th} infestation period.

However, Huxham *et al.* (2001) while working on respiratory rates in the marine periwinkle *Littorina littorina* infected with three species of Digenea obtained contradictory results. According to paired 't' test showed no significant decrease between the mean oxygen uptake of the infected and uninfected snails for any of the three species of parasites from marine environment. Shinagawa *et al.* (2001) while working on effect of trematode infection on metabolism and respiratory activity in the freshwater snail, *Semisulconspira libertina* are of the opinion that infection with the marine cercaria significantly increased the oxygen consumption rate in large sized animals. The present snail *L. acuminata* selected for respiratory metabolism studied having 20±2 mm shell length with fully developed rediae with mature cercariae in it, showed increased rate of respiration throughout patency period. Von Brand *et al.* (1948) and William and Gilbertson (1983) have opined that the increased oxygen consumption may be a cause of feeding increasd, as it is related to feeding frequency, like other basal metabolic parameters such as the heart rate.

Maintenance metabolism in snails (measured as oxygen consumption) as in most animals is apparently the most important rout of daily energy expenditure (Studier et al. 1975). However the results of early study are contradictory and suggested that schistosome infection had little effect on host respiration (Malek and Cheng, 1974), decreased oxygen consumption has been reported in *S. mansoni* infected *B. alexandrina* during the prepatent period (Ishak et al., 1975). Subsequent studies with tissue homogenates for *B. alexandrina* and *B. truncatus* demonstrated reduced oxidation of exogenously supplied tricarboxylic acid cycle, intermediate and in inhibition of respiratory chain enzyme system in infected snails. Consistent with decreased oxygen consumption, several investigations have demonstrated elevated glycolytic rate and glycolytic enzymes in parasitized host (Marshall et al., 1974).

Various factors which are expected to significantly affect oxygen consumption such as intrinsic and extrinsic factors to the daily energy budget of the species. The snail *L. acuminata* when get invaded by larval trematode parasites in its body naturally, there will be an increased budgetary demand and utilization for larval parasitic growth and development may be the major cause to have increased rate of oxygen consumption during patency period of the snail *Lymnaea*.

The pond snail *L. acuminata* is widely distributed through-out the tropical regions of the country India, and the physiological response of this species to larval trematode infection is not well documented. Hence the present small attempt does not serve even as a mite in the field of host parasite physiological relationship.

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