[Jap. J. Parasit., Vol. 20, No. 1, 62-66, 1971]

Paragonimus ohirai, Miyazaki, 1939: Oxygen Debt and the Influence of Various Carbohydrates on Respiration*

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(Received for publication; December 17, 1970)

Introduction

Although adult flukes generally have been classified as facultative anaerobes (Bueding, 1949; von Brand, 1952; Goil, 1958), little work has been done on the influence of carbohydrate substrates on their respiration (Vernberg, 1963). Studies have been performed on the influence of glucose on the respiration of *Schistosoma mansoni* (Bueding, 1950); *Paragonimus westermani* and *S. japonicum* (Shimomura, 1959); and *P. ohirai* (Bruce *et al.*, 1971). The effects of other carbohydrates on adult trematode respiration have not been reported.

Some animals, after having been exposed to an anaerobic enviroment, show increased oxygen consumption when they are returned to oxygenated surroundings (von Brand, 1966). This phenomenon, known as repayment of oxygen debt, has been studied in only a few species of tramatodes, including *S. mansoni* (Bueding, 1950), *Gynaecotyla adunca* (Hunter and Vernberg, 1955), and *P. westermani* (Read and Yogore, 1955).

The purpose of this study was to determine the influence of carbohydrates or carbohydrate derivatives on the respiratory rates of adult *P. ohirai* at 1 and 25 hr after collection. In addition, the repayment of oxygen debt, following a 30-min anoxic period, was investigated for worms of the same age.

Materials and Methods

Thirty adult *P. ohirai* were collected from albino rats (406th Medical Laboratory inbred strain) which had each been exposed previously to 30 metacercariae obtained from *Sesarma dehaani* crabs. Worms were prepared for each respiration study according to the technique of Bruce *et al.* (1971), and placed into a buffer system (pH 7.7) composed of the following: 0.137 M NaCl, 0.0085 M KCl, 0.0003 M $CaCl_2$, 0.005 M $MgCl_2$, and 0.006 M Na_3PO_4 (Bueding, 1950). The oxygen uptake of the worms was measured by the direct Warburg method (Umbreit *et al.*, 1964). Five worms were placed into the main compartment of a 15-ml Warburg flask with 3.0 ml of buffer. Filter paper fans and 0.2 ml of 20% KOH were placed in the center well for CO_2 adsorption. Respiration was measured at 37°C in a gas phase of air (20% O_2) for 1 hr. Respiration measured at 1 hr after collection was designated as oxygen consumption by adults immediately after collection. Following this trial, the worms were removed from the reaction vessels, washed, and maintained in sterile buffer for 24 hr (4°C). At the end of this period,

* Experiments were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council. Financial support for this study was provided by the U. S. Army Medical Research and Development Command, Office of the Surgeon General, Washington, D.C.

respiration was again measured by the same procedure. These worms were designated as aged adults (25 hr after collection).

Ten experiments were conducted (Exp. I-X). In Experiment I respiration of both immediate and aged worms were determined in standard buffer without added carbohydrate. In Experiments II-IX the respiration of immediately collected and aged adult worms was measured in buffer containing 0.004 M of one of the following carbohydrates: glucose, fructose, galactose, mannose, glucosamine, maltose, lactose, and mannitol, respectively. Two trials were made with each substrate at both 1 and 25 hr after collection.

In Experiment X, two trials were performed to study repayment of oxygen debt. The respiration of adult worms was first measured aerobically in buffer without added carbohydrate for 30 min. The worms were then transferred to a second set of flasks and manometers in which the gas phase was 95 % N_2–5 % CO_2. The system was flushed for 10 min with the N_2-CO_2 gas and after 30 min in the anaerobic environment the worms were again returned to their original flasks. After a 10-min equilibration period the oxygen consumption was measured for several 30-min post-anaerobic periods. In each trial, measurements were made for five postanaerobic periods with worms at 1 hr after collection and for four postanaerobic periods with aged worms.

Following the measurement of O_2 consumption, each worm was dried in an individual aluminum weighing pan at 100°C for 18–24 hr, in order to express all data as μl O_2 consumed/hr/mg of dry tissue. In summarizing results for tabular presentation, data from each trial were averaged. Student's "T" tests (Snedecor, 1956) were used to judge significant differences.

Results

The effects of various substrates on the respiratory rate of *P. ohirai* are shown in Table 1. Only glucosamine significantly increased the respiratory rate of worms at 1 hr after collection, compared with the respiratory rate in buffer only. For the same time interval, glucose, lactose, and mannose significantly decreased the QO_2. Maltose, fructose, and mannitol had no effect. At 25 hr after collection of worms, the addition of every one of the eight substrates resulted in a significant increase in the QO_2 value when compared with that obtained in buffer without added substrate.

Table 1 Oxygen uptake of adult *Paragonimus ohirai* in various 0.004 M substrates*

Experiment No.	Substrate	QO_2 (μl O_2/mg dry wt/hr)	
		1 hr after collection	25 hr after collection
I	Buffer only	2.132±0.716	0.694±0.348
II	Glucose	1.418±0.452**	1.193±0.427***
III	Glucosamine	2.847±0.746***	2.408±0.586***
IV	Maltose	1.623±0.240	2.330±0.640***
V	Fructose	2.003±0.496	1.732±0.732***
VI	Lactose	1.170±0.408**	2.226±0.385***
VII	Mannitol	1.802±0.401	1.618±0.585***
VIII	Mannose	1.168±0.381**	1.075±0.467***
IX	Galactose	2.094±0.550	2.019±0.492***

* The experiments were performed with 5 adult worms/flask containing 3.0–5.0 ml of buffer composed of 0.137 M NaCl; 0.0085 M KCl; 0.0003 M $CaCl_2$; 0.005 M $MgCl_2$; and 0.006 M Na_3PO_4 (pH 7.7) (Bueding, 1950). Two trials were made in each experiment. Each QO_2 value represents the average of 6 to 15 flasks.

** Significant decrease (P>.90) when compared with buffer only.

*** Significant increase (P>.90) when compared with buffer only.

Table 2 Oxygen uptake of adult *Paragonimus ohirai* measured for 30-min pre- and postanaerobic periods*

Time period	Hr after collection	Trial	QO_2 ($\mu l\ O_2$/mg dry wt/hr)	Average QO_2
Preanaerobic	1	1	3.606±1.984	2.778±1.880
		2	1.949±1.689	
	25	1	1.195±0.674	1.494±0.567
		2	1.794±0.273	
Postanaerobic I			results variable**	
Postanaerobic II	1	1	1.329±0.352	1.264±0.479
		2	1.195±0.630	
	25	1	2.568±0.470***	2.527±0.586***
		2	2.519±0.796	
Postanaerobic III	1	1	1.730±0.408	1.252±0.727
		2	0.775±0.688	
	25	1	1.487±0.442	2.066±0.704***
		2	2.645±0.080***	
Postanaerobic IV	1	1	1.432±0.475	1.111±0.596
		2	0.786±0.587	
	25	1	1.271±0.553	1.234±0.421
		2	1.197±0.365	
Postanaerobic V	1	1	0.822±0.587	1.074±0.640
		2	1.326±0.700	

* The experiments were performed with 5 adult worms/flask containing 3.3–5.0 ml of buffer composed of 0.137 M NaCl ; 0.0085 M KCl ; 0.0003 M $CaCl_2$; 0.005 M $MgCl_2$; and 0.006 M Na_3PO_4 (pH 7.7) (Bueding, 1950). Three trials were made in each experiment.

** Some flasks during postanaerobic period I showed positive readings while others were negative.

*** Significant increase (P>.90) when compared with the QO_2 of the preanaerobic period.

The results of studies on repayment of oxygen debt by *P. ohirai* are presented in Table 2. In both trials, the QO_2 values obtained in the first 30-min postanaerobic period were variable (e. g., some flasks showed a positive QO_2 value while others showed a negative value). Because of this variation, the average QO_2 values for the first postanaerobic period were not calculated. Freshly collected worms showed no repayment of oxygen debt in any of the postanaerobic periods. The respiratory rate of these worms was not significantly different from that observed during the preanaerobic period. Repayment of oxygen debt was observed with aged worms in postanaerobic periods II and III in trials 1 and 2, respectively. The average QO_2 value showed a significant increase in respiration for both postanaerobic periods II and III. Based on the QO_2 value of the preanaerobic period, the quantitative replacement of the oxygen debt incurred was 69 % and 38 %, for postanaerobic periods II and III, respectively. This indicates that the oxygen debt was repayed by worms at 25 hr after collection.

Discussion

The QO_2 values for worms at 1 and 25 hr after collection are comparable to those

reported previously (2.132 and 0.694 μl O_2/mg dry wt/hr, respectively, Bruce *et al.*, 1971). The QO_2 values obtained with freshly collected and aged worms when 0.004 M glucose was added to the buffer system (1.418 and 1.193 μl O_2/mg dry wt/hr, respectively) are also comparable to those reported previously (1.466 and 1.153 μl O_2/mg dry wt/hr, respectively, Bruce *et al.*, 1971). Adult *P. ohirai* in buffer consumed significantly less oxygen at 25 hr after collection than worms at 1 hr after collection. This may reflect a depletion of substrates required for energy metabolism in aged worms, since when exogenous glucose was provided, no difference was found between freshly collected and aged worms.

The decrease in the QO_2 value for worms at 1 hr after collection when glucose, lactose, or mannose was added to the buffer system, might have been due to the "Crabtree" effect. This effect is produced by the increased competition of the anaerobic glycolytic process for inorganic phosphate and possibly pyridine nucleotides, leaving less for oxidative phosphorylation reactions (West and Todd, 1964; Fruton and Simmonds, 1963). Bueding (1950) found a slight increase in the QO_2 value of paired *S. mansoni* adults in the presence of glucose (8.7 vs. 6.0 with glucose present and absent, respectively). Read and Yogore (1955) reported a QO_2 value of 0.74–0.86 for *P. westermani* in Krebs-Ringer phosphate containing 0.01 M glucose, while Shimomura (1959) reported a value of 2.8. In the present study a significant increase was found in the QO_2 of freshly collected worms when glucosamine was added to the buffer. Since the effects of glucose and glucosamine on respiration of these worms are different (i. e., inhibition and stimulation, respectively), this may suggest that different enzymes are responsible for the phosphorylation and utilization of these two substrates. Bueding *et al.* (1954) have shown that in *S. mansoni*, the phosphorylation of glucosamine and of glucose are catalyzed by two different enzymes.

The addition of each of the substrates tested significantly increased the QO_2 value of aged worms compared with the value obtained in buffer only. This might reflect the replacement of depleted energy substances. When glucose, glucosamine, fructose, mannitol, mannose, and galactose were added to the buffer system, the QO_2 values at 25 hr after collection were comparable with those values obtained for each substrate at 1 hr after collection. This suggests that these substrates are readily taken up and utilized as energy sources by aged worms. With maltose and lactose, the QO_2 values at 25 hr after collection were significantly higher than those at 1 hr after collection, indicating that greater stimulation of respiration was occurring with these substances.

Most animals can obtain some energy anaerobically and then later oxidize the products as an oxygen debt. This concept is highly relative and the magnitude of debt which is repayed is variable. Animals which are sensitive to anaerobiasis usually pay off some debt; those which tolerate lack of oxygen may pay off a small debt or none at all (Prosser and Brown, 1961).

In this study, it was found that aged worms repayed an oxygen debt incurred by a 30-min anaerobic exposure while freshly collected worms did not. The variable results found in postanaerobic period I might be the result of insufficient equilibration during the equilibration period. The absence of oxygen debt repayment at 1 hr after collection might have been due to the failure of these worms to accumulate the end products of anaerobic metabolism within the tissues, since these end products serve as necessary substrates for increased oxygen consumption when respiratory rebound occurs (von Brand, 1966). It has beed reported that *Schistosoma mansoni* (Bueding, 1950), and *Gynaecotyla adunca* (Hunter and Vernberg, 1955) show no evidence of accumulating an oxygen debt during an anoxic period. Also, in the case of *S. mansoni*, practically all the anaerobically consumed carbohydrate may be accounted for by acids excreted into the medium (Bueding, 1950).

Increased QO_2 values were found in the

second and third postanaerobic periods of worms at 25 hr after collection when compared with the preanaerobic QO_2 values. This higher rate might have been the result of the rapid alteration of anaerobic end products (which are often toxic) to nontoxic substances, either by total oxidation or by partial resynthesis to carbohydrate (von Brand, 1966). Overpayment of oxygen debt occurs in *Paragonimus westermani* (Read and Yogore, 1955). The QO_2 values reported following a 30-min anoxic period increased to 1.72–5.06 and 1.58–2.75 in *P. westermani* during the first and second 30-min postanaerobic periods, respectively, compared with preanaerobic values of 0.74–0.86.

Abstract

The respiration of adult *Paragonimus ohirai* was measured at 1 and 25 hr after collection in phosphate buffered saline or buffer containing 0.004 M glucose, glucosamine, maltose, fructose, lactose, mannitol, mannose, or galactose. At 1 hr after collection, the addition of glucose and lactose to the medium significantly decreased the respiratory rate compared to buffer only, while the addition of glucosamine resulted in a significant increase in the QO_2 value. The other substrate did not change the respiratory rate. At 25 hr after collection, all substrates tested produced a significant increase in QO_2 value when compared with that obtained in buffer only.

In two trials, worms were pre-exposed to a 30-min anaerobic period. Results of these studies indicated that, at 25 hr after collection, complete repayment of oxygen debt occurred in the second and third 30-min postanaerobic periods, based on the QO_2 values obtained during a preanaerobic period. No repayment of oxygen debt was observed at 1 hr after collection.

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