PARAGONIMUS WESTERMANI : INFLUENCE OF CARBOHYDRATES ON RESPIRATION OF FOUR GEOGRAPHIC STRAINS WITH COMPARISON TO *P. OHIRAI* AND *P. ILOKTSUENENSIS*†

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INTRODUCTION

Three species of *Paragonimus (P. westermani, P. ohirai,* and *P. iloktsuenensis)* are commonly found throughout the Asian area. Although some attempts have been made to study the effect of glucose on the respiration of various medically important trematodes (Bueding, 1950; Shimomura, 1959; Bruce *et al.*, 1971a, b, c; Ruff *et al.*, 1971), little is known about the effects of other carbohydrates on adult trematode respiration (Bruce *et al.*, 1971d). Also, there is little information available concerning the comparative respiration of different species of *Paragonimus*.

Bruce *et al.*, (1971c) have emphasized the difficulties in maintaining the complete life cycle of *P. westermani* in the laboratory and have suggested that other species, which can be more easily maintained, might serve as a model for physiological studies of paragonimiasis, if the physiology of the different species were the same or similar.

The purpose of this study was to determine if four geographic strains of *Paragonimus westermani* would respond similarly to exogenous carbohydrate substrates. Additional comparisons to the respiration of *P. ohirai* and *P. iloktsuenensis* were made. Respiration was measured in buffer containing added carbohydrates at 1 and 25 hours after collection.

MATERIALS AND METHODS

Adult *P. westermani* were obtained from cats[1] which had been previously exposed to 50 metacercariae obtained from naturally infected crabs. *Paragonimus westermani* metacercariae of the Japanese strain were collected from *Potamon dehaani* crabs; the Taiwanese strain from *P. dehaani* and *Eriocheir japonicus* crabs, the Philippine strain from *Potamon* sp. crabs and the Korean strain from crayfish, *Cambaroides similis*.

Thirty adult worms were prepared for each respiration study according to the technique of Bruce *et al.*, (1971c) and placed in a sterile aerated 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 $MgCl_2$,

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[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

and 0.006 M Na_3PO_4, (Bueding, 1950). Four or five of the 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 centre well for CO_2 absorption. The oxygen uptake of the worms was measured by the direct Warburg method (Umbreit *et al.*, 1964) at 37°C in a gas phase of air (20% 0_2) for 1 hour. Respiration measured at 1 hour after collection was designated as oxygen consumption by freshly collected worms. Following this trail the worms were removed from the reaction vessels, washed, and maintained in sterile buffer for 24 hours (4°C). At the end of this period, respiration was again measured by the same procedure, these worms being designated as aged adults (25 hours after collection). Four experiments were conducted with each of the four strains. Respiration of freshly collected and aged adult worms was measured in buffer only and buffer containing 0.004 M glucose, glucosamine, lactose, or mannose.

Following the measurement of O_2 consumption, each worm was dried at 100°C for 18-24 hours in separate, individually weighed aluminum pans. Dry weights were then taken in order to calculate the µl O_2/mg dry weight per hour. The Student's "t" test (Snedecor 1956) was used to determine significant differences. All data were averaged for each experiment for presentation in tabular form.

RESULTS

The respiration of the four geographic strains of adult *Paragonimus westermani* in a phosphate buffer containing various carbohydrates is summarized in Table 1. The results of earlier studies with *P. ohirai* and *P. iloktsuenensis* (Bruce *et al.*, 1971d and Ruff *et al.*, 1971, respectively) are also shown for comparison.

The design of the experimental model made possible the detection of two types of effects on respiration: (1) a substrate effect detected by comparison of the QO_2 value for each substrate with the value obtained for worms of the same age in buffer only; and (2) an age effect determined by comparison of the QO_2 values obtained for worms in the same substrate at 1 and 25 hours after collection. Added glucose had no effect upon the respiration of either the immediately collected or aged adult *P. westermani*. The three remaining carbohydrates (glucosamine, lactose and mannose) had different effects upon the four strains of *P. westermani* studied, depending on the strain and age of the worm. In all cases where the carbohydrate influenced the respiration, a significant increase was noted compared with the QO_2 values in buffer only. Glucosamine increased respiration for freshly collected adults of the Philippine and Korean strains and aged adults of the Taiwanese strain. Lactose increased the respiration of freshly collected adults of the Philippine strain. Mannose increased the QO_2 value of freshly collected adults of the Taiwanese strain and adults of the Japanese strain at both 1 and 25 hours after collection.

In most cases there were no differences between the QO_2 value of aged worms and the QO_2 value of freshly collected worms in the same substrate. An age dependent decrease was found, however, with glucose in the Philippine strain and with glucosamine in the Philippine and Korean strains.

DISCUSSION

It has been pointed out by Bruce *et al.*, (1971c) that certain free-living larval stages of trematodes may initially obtain a portion of their energy requirements from the anaerobic glycolysis of glycogen to pyruvate or lactate.

Table 1

Influence of carbohydrates on respiration of different species and strains of adult *Paragonimus*[1].

Species	Strain	Dry weight	Hours after collection	QO_2 (µl O_2/mg dry wt/hr) Substrate				
				Buffer	Glucose	Glucosamine	Lactose	Mannose
P. westermani[2]	Japanese	6.280 ± 2.163	1	1.358 ± 0.437	1.006 ± 0.307	1.171 ± 0.319	1.408 ± 0.574	2.144 ± 0.656[5]
			25	0.811 ± 0.452	0.661 ± 0.239	0.780 ± 0.283	1.313 ± 0.503	2.284 ± 0.626[5]
P. westermani[2]	Philippine	7.900 ± 1.158	1	0.729 ± 0.063	0.877 ± 0.063	0.905 ± 0.077[5]	1.030 ± 0.141[5]	0.701 ± 0.656
			25	0.539 ± 0.425	0.487 ± 0.077[6]	0.628 ± 0.045[6]	0.616 ± 0.303	0.811 ± 0.158
P. westermani[2]	Taiwanese	5.880 ± 1.806	1	0.734 ± 0.185	0.658 ± 0.276	0.987 ± 0.432	0.933 ± 0.355	1.581 ± 0.586[5]
			25	0.543 ± 0.244	0.666 ± 0.176	1.273 ± 0.308[5]	0.471 ± 0.230	0.851 ± 0.268
P. westermani[2]	Korean	3.550 ± 1.373	1	1.465 ± 0.510	1.587 ± 0.859	2.310 ± 0.345[5]	1.440 ± 0.428	1.695 ± 0.428
			25	1.083 ± 0.470	0.933 ± 0.692	0.945 ± 0.566[6]	1.222 ± 0.335	1.488 ± 0.676
P. ohirai[3]	Japanese	2.782 ± 0.968	1	2.132 ± 0.716	1.418 ± 0.452[5]	2.847 ± 0.746	1.170 ± 0.408[5]	1.168 ± 0.381[5]
			25	0.694 ± 0.348[6]	1.193 ± 0.427[5]	2.408 ± 0.586[5]	2.226 ± 0.385[5,6]	1.075 ± 0.467[5]
P. iloktsuenensis[4]	Taiwanese	1.784 ± 0.148	1	1.914 ± 0.606	2.150 ± 0.627	-	-	-
			25	5.081 ± 1.087[6]	4.272 ± 1.184[6]	-	-	-

[1] The experiments were performed with 4 or 5 adult worms/flask containing 3.0 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 (pH7 .7) (Bueding, 1950). The worms were maintained at 37°C for 1 hr. Each QO_2 value is the mean of 6 flasks. The data are expressed as the mean ± standard error of respiration rate per mg of dry tissue weight per hr.

[2] Results from present study using 0.004 M carbohydrate.

[3] Results from Bruce *et al.*, (1971d) using 0.004 M carbohydrate.

[4] Results from Ruff *et al.*, (1971) using 0.001 M carbohydrate.

[5] Significant difference ($P<0.05$) when compared to buffer only.

[6] Significant difference ($P<0.05$) when compared to the QO_2 value of freshly collected worms in the same substrate.

As aging progresses and these energy reserves are depleted, an increasing portion of the energy requirements is met by aerobic metabolism. If the same relationship exists in the parasitic adult, then an indication of the relative amounts of energy reserves between species or strains can be obtained by a comparison of the QO_2 values of freshly collected and aged worms in buffer without added substrate. Three different aging effects would be possible: (1) No significant difference in the QO_2 value of freshly collected and aged adults which would indicate large energy reserves; (2) An increase in the QO_2 value with aging which would suggest moderate energy reserves (i.e., a shift from anaerobic to aerobic metabolism); and (3) A decrease in the QO_2 value with aging which would indicate only slight initial energy reserves (i.e., depletion of both anaerobic and aerobic endogenous substrates). On this basis, comparison of the results of oxygen consumption of adult *Paragonimus* in buffer without substrate at 1 and 24 hours after collection would suggest that *P. westermani* has large endogenous energy reserves, *P. iloktsuenensis* moderate energy reserves, and *P. ohirai* only small energy reserves. To date, most studies of the aging effect on the respiration of free-living trematode larvae have indicated large or moderate energy reserves (i.e., no change and/or increase in QO_2 value with aging). These studies have reported increased oxygen uptake with aging for *P. ohirai* miracidia (Bruce *et al.*, 1971c), *Schistosoma mansoni* cercariae (Bruce *et al.*, 1971b), and the Japanese and Formosan strains of *S. japonicum* miracidia (Bruce *et al.*, 1971a). No change in the QO_2 value with aging was reported for miracidia of the Philippine strain of *S. japonicum* (Bruce *et al.*, 1971a).

Further evidence for strain and species differences in endogenous energy reserves was found in the effect of substrates on oxygen uptake. The response of both freshly collected and aged adults of the four geographic strains of *P. westermani* was somewhat varied but, in general, the addition of carbohydrate resulted in only a slight increase (less than twofold) or no change in the QO_2 value. This would indicate either little or no exogenous substrate utilization and/or little or no substrate uptake by the worm. Likewise, the addition of glucose produced no change in QO_2 value with *P. iloktsuenensis* (Ruff *et al.*, 1971).

The response of *P. ohirai* to substrate addition was considerably different. The addition of glucose, lactose, and mannose to freshly collected worms resulted in a decrease in the QO_2 value compared with that obtained in buffer only (Bruce *et al.*, 1971d). This response probably reflects a shift of a portion of aerobic metabolism back to anaerobic metabolism due to increased availability of substrate. This may be connected with the "Crabtree" effect in which there is competition by the anaerobic glycolytic process for inorganic phosphate and possibly pyridine nucleotides, leaving less for oxidative phosphorylation reactions (Fruton and Simmonds, 1963; West and Todd, 1963). With aged *P. ohirai*, the addition of glucose, glucosamine, lactose, and mannose resulted in an increase in the QO_2 value compared with the oxygen uptake in buffer only. This would suggest a replacement of depleted anaerobic and aerobic endogenous energy reserves by the exogenous substrate.

In the present study the QO_2 values obtained for freshly collected adult *P. westermani* tested in buffer containing 0.004 M glucose are similar to those values reported previously by Read and Yogore (1955) and Shimomura (1959) for *P. westermani* tested in Krebs-Ringer phosphate buffer containing 0.01 M glucose.

The results discussed above show that there are distinct differences in the respiratory response of *P. westermani*, *P. ohirai*, and *P. iloktsuenensis* to aging and to the addition of certain exogenous carbohydrate substrates. Slight, but significant, differences were found even among the four geographic strains of *P. westermani* studied. The findings of Yoshimura (1969) and Yoshimura *et al.*, (1969) that these three species of *Paragonimus* have distinct differences in the electrophoretic patterns of whole body proteins, would suggest that there might be other important physiological and metabolic differences as well.

In view of these observed differences in the metabolism of various strains and species of *Paragonimus*, it is recommended that great caution be exercised in utilizing any one strain or species as a model for physiologic studies of paragonimiasis in general, unless it has first been established that the response of all species is the same for the particular aspect of metabolism being studied.

SUMMARY

The respiration of adult *Paragonimus westermani* from Japan, the Philippines, Taiwan, and Korea was studied. The oxygen uptake of worms at 1 and 25 hours after collection was measured in buffer only and in buffer plus 0.004 M glucose, glucosamine, lactose, or mannose. An increase in the QO_2 value was found with some geographic strains of *P. westermani* when glucosamine, lactose, or mannose was added to the buffer, indicating some utilization of these substrates for respiration. Comparison with the effects of these substrates on *P. ohirai* and *P. iloktsuenensis*, reported previously, indicated that there are physiological differences among these three species.

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