Respiratory Physiology of a Facultative Anaerobe, Romanomermis culicivorax[1]

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Abstract: The respiratory physiology of postparasitic larval and adult *Romanomermis culicivorax* was studied by manometric and polarographic methods. Endogenous respiration rates were relatively low and unaffected by postemergent development. The respiratory quotient (RQ) of larvae and young adults was 0.4 but increased to 0.7 about 3 wk after emergence. Exogenous glucose (0.02 m*M*) had no effect on Q_{O_2} or RQ. Respiration of adults and larvae was completely inhibited by KCN (1 m*M*) but not by NaN_3 (1 m*M*) or 2,4-DNP (0.1 m*M*). The nematodes survived exposure to cyanide (1 m*M*) for 18 h. Carbon dioxide (10%) inhibited respiration. The postparasitic stages of the nematode were mixed respiratory regulators-conformers. Exposure to anaerobic conditions resulted in an increased postanaerobic oxygen consumption which persisted for 3.5 h. The experiments confirmed that the postemergent stages of the nematode are facultative anaerobes. *Key words:* oxygen tension, carbon dioxide, respiratory quotient, O_2 debt, Mermithidae, Nematoda. Journal of Nematology 14(2):226-231. 1982.

The mermithid nematode *Romanomermis culicivorax* is a promising biological control agent of mosquitoes (8,17). Most field tests have utilized preparasites as inoculum, but recent field applications using postparasites have met with good success (19). There is a substantial amount of information on the physiology of the preparasites as related to environmental limitations on their usefulness (8,17), but comparatively little is known about the physiology of postparasites. In a recent series of experiments on the gaseous requirements for postemergent development, the nematodes completed development under anaerobic atmospheres containing carbon dioxide (6). The results of these experiments, along with the anoxic nature of their environment, suggested that the postparasitic stages of *R. culicivorax* function as facultative anaerobes. The purpose of this study was to determine the nature of the respiratory physiology of the nonparasitic stages of the nematode.

MATERIALS AND METHODS

Nematode culture: Romanomermis culicivorax cultures were maintained in an autogenous strain of *Culex pipiens* using Platzer and Stirling's (9) modification of the procedures of Petersen and Willis (7). Postparasitic larvae (L_3) were collected as they emerged from the host mosquito larvae and cleaned by Baermannization through two layers of cheesecloth and exhaustive washing with tap water. Nematodes were collected on a 32 mesh screen, backwashed into a 1-liter plastic container, then washed with a stream of water and collected again on the screen. This process was repeated 8 to 10 times for nematodes that were used in the experiments.

Endogenous respiration: Respiration was determined by manometry (Gilson Differential Respirometer) and polarography (oxygen electrode, Yellow Springs Instrument Model 53). Nematodes (18–25 mg dry wt) were placed in 2.0 ml of bathing medium (Spring Water, Arrowhead) in 15-ml double sidearm flasks with the center well containing folded filter paper and 0.2 ml 20% KOH. Similar flasks without KOH were used for determination of CO_2 production (13). Carbon dioxide production (Q_{CO_2}) was expressed as μl CO_2 produced · h^{-1} · mg dry wt^{-1}. Oxygen uptake was calculated from the slope of the line fitted to the plot of total oxygen uptake vs. time using the least mean squares method and expressed as μl O_2 consumed · h^{-1} · mg dry wt^{-1} (Q_{O_2}). Respiratory quotients (RQ) were calculated as Q_{CO_2}/Q_{O_2}. All experiments were performed at 27 C and gas volumes corrected to standard temperatures and pressure. Glucose or inhibitors were added from the sidearms. Control flasks consisted

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of bathing medium without nematodes. When the O_2 electrode was used, the nematodes (30–50 mg dry wt) were placed in 5.0 ml bathing medium and O_2 consumption measured for 15 min. Dry weights were determined by collecting the nematodes on a 0.45-μm pore filter washing with distilled water to remove salts, then drying to a constant weight in a vacuum desiccator containing $CaSO_4$.

Influence of oxygen concentration: Respirometer flasks were prepared with a vented plug in one sidearm. Mixtures of 1.0% CO_2 and various O_2 and N_2 concentrations were prepared in a four-tube single outlet rotameter. The mixtures were passed through the respirometer manifold and the flasks at a flow rate of 1.0 liter per min. Fifteen minutes were allowed for gaseous equilibration of the bathing medium. The valves and vents were closed and after an additional 15 min, equilibration respiration was determined. The process was repeated with different groups of nematodes for all O_2 concentrations tested. Several control flasks were included to correct for O_2 leakage from tygon tubing in the respirometry system at 100% O_2 (Gilson Medical Electronics Bulletin).

Effect of carbon dioxide: Mixtures of 21% O_2, various CO_2 concentrations, with the balance N_2 were prepared in the rotameter and passed through the bathing medium containing the nematodes at 1.0 liter per min for 10 min. Oxygen consumption was measured with the oxygen electrode for 15 min.

Oxygen consumption and respiratory quotient during postparasitic development: To determine the effects of postparasitic development on oxygen consumption and respiratory quotient, nematodes were maintained under conditions simulating our standard cultural conditions for *R. culicivorax*. Two milliliters of postparasites (packed volume after 5 min centrifugation @ 250 × G) were placed in 500-ml plastic containers with 300 ml spring water. The containers were sealed with plastic lids and held at 27 C. Oxygen concentrations were determined in replicated containers using a Beckman Model 1008 Oxygen electrode. After 9, 16, 23, and 30 days the containers were opened, the oxygen concentration was measured quickly, the nematodes were removed and washed, and O_2 consumption determined immediately with the Yellow Springs oxygen electrode.

Postanaerobic oxygen consumption: To determine the effect of postemergent development under aerobic and anaerobic atmospheres on respiration, 2.0 ml postparasites (packed volume as above) were placed in 500-ml plastic containers containing 300 ml deoxygenated spring water. The containers were sealed with plastic lids fitted with tygon hoses through which air (aerobic) or a 95% N_2-5% CO_2 mixture (anaerobic) were passed at a constant flow of 80 ml per min. The containers were hooked in series with the gas inlets below the surface of the water and the exhaust above the water. After 8 and 15 days at 27 C, the nematodes were removed and washed and O_2 consumption determined immediately using the O_2 electrode.

Statistical analysis: Three to five replications were used for all experiments which were repeated two or more times. Analysis of variance and Duncan's multiple-range tests were utilized to determine statistical significance.

RESULTS

Endogenous respiration: The relationship between dry weight and Q_{O_2} was linear over the range of weights used for all experiments. Q_{O_2} and RQ were constant over a period of 12 h of continuous measurement in the respirometer. No respiration was observed in the control flasks nor was a significant residual respiration found after removal of the nematodes in any of the experiments.

Endogenous respiration rates and RQ were similar for *R. culicivorax* 2 and 5 days postemergence and the addition of glucose did not alter either the Q_{O_2} or RQ.

Oxygen utilization and RQ of postparasitic *R. culicivorax* at various stages of development are presented in Table 1. Dissolved O_2 in the spring water in the containers with postparasites declined from 8.2 $mg \cdot L^{-1}$ (saturated with air) at the start of the experiment to 4.0 $mg \cdot L^{-1}$ within 48 h and remained at this concentration for the remainder of the experiment. Q_{O_2} increased 51% between the 2nd and 9th day post-

Table 1. Endogenous respiration of *Romanomermis culicivorax* during postemergent development.*

Days post-emergence	Stage	Q_{O_2}†	RQ‡
2	L_3	0.961 A	0.447 A
9	L_3	1.480 B	.....
16	Adult	1.194 B	0.462 A
23	Adult	1.275 B	0.710 B
30	Adult	1.206 B	0.750 B

*Mean of three replicates in spring water. Values followed by different letters are significantly different ($P \leq 0.05$).

†Q_{O_2} = μl O_2 consumed · h^{-1} · mg dry wt^{-1}.

‡RQ = Q_{CO_2}/Q_{O_2} where Q_{CO_2} = μl CO_2 produced · h^{-1} · mg dry wt^{-1}.

emergence but remained constant thereafter. There was a significant shift in the RQ from 0.45 prior to 25 days to 0.73 at later times. Development of the nematodes appeared normal for these conditions; molting occurred between 9 and 16 days postemergence (6).

The respiratory inhibitors NaN_3 (1 m*M*) and 2,4-dinitrophenol (0.1 m*M*) did not inhibit respiration of L_3 (3 days postemergence); KCN (1 m*M*) severely inhibited oxygen uptake by 95%, but the nematodes did not appear to be physiologically damaged even after overnight (18 h) exposure. Respiration returned to normal levels after removal of the inhibitor by repeated washes with spring water.

Influence of carbon dioxide: Up to 5.0% CO_2 had no adverse effect on oxygen utilization of 6-day-old postparasites, but higher concentrations depressed respiration significantly (Fig. 1). At 10%, 20%, and 30% CO_2 the Q_{O_2} was about 86%, 64%, and 56%, respectively, of the value obtained in air (0.03% CO_2). Similar responses were seen with freshly emerged nematodes.

Response of R. culicivorax to various oxygen tensions: The influence of O_2 tension on respiration varied with stage of development. Larvae (4 days postemergence) and adults (25 days postemergence) responded in a similar manner. Respiration was constant at O_2 concentrations from 5 to 20% but increased at concentrations above 20% and fell off rapidly with concentrations below 5% (Fig. 2A and C).

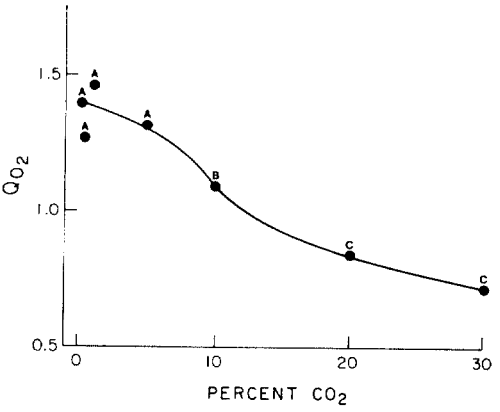

Fig. 1. Effect of carbon dioxide on respiration of *Romanomermis culicivorax* L_3 (6 days postemergence). Oxygen uptake determined polarographically in spring water equilibrated with N_2, 21% O_2, and various concentrations of CO_2. Each point is the mean of three replications. Points followed by different letters are significantly different ($P \leq 0.05$).

Ten-day postemergent larvae were affected by O_2 concentrations of 20% and less in the same way as the other stages tested, but

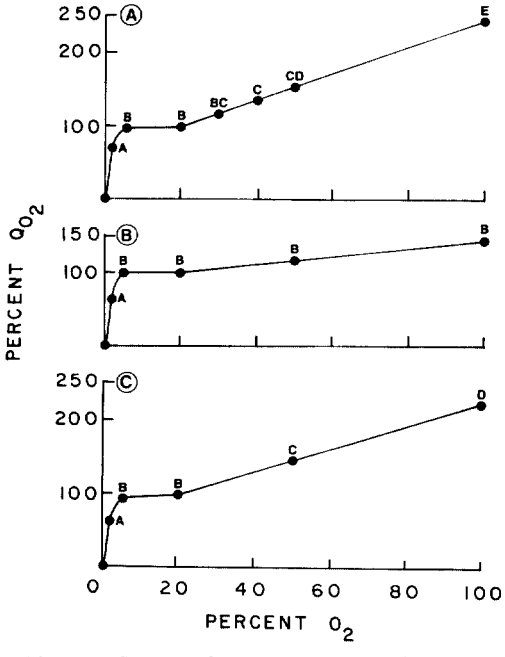

Fig. 2. Influence of oxygen concentration on respiration of postparasitic *Romanomermis culicivorax*. Respiration determined manometrically in spring water equilibrated with N_2, 1% CO_2, and various concentrations of O_2. Data expressed as percent of Q_{O_2} at 20% O_2. Each point is a mean of three replications. Points followed by different letters are significantly different ($P \leq 0.05$). A) L_3 4 days postemergence. B) L_3 10 days postemergence. C) Adults 24 days postemergence.

there was no significant increase in respiration at greater O_2 levels (Fig. 2B).

Postanaerobic oxygen consumption: The influence of incubation under aerobic and anaerobic conditions on subsequent respiration in water saturated with a mixture containing 21% O_2 is given in Table 2. Nematode development was similar to that seen in experiments on agar (6). In the presence of O_2 the larvae molted to adults between the 8th and 15th days postemergence but did not molt by day 15 in the absence of oxygen. Nematodes held under aerobic conditions after emergence utilized oxygen at the same rate regardless of stage of development. After anaerobic incubation there was a considerable stimulation of respiration when given access to O_2 (49 to 137% greater). This increase was evident immediately after removal from anaerobic conditions and was maintained for at least 3.5 h. There was no significant differences in the postanaerobic O_2 consumption between nematodes held 8 and 15 days without O_2.

DISCUSSION

Oxygen uptake by postparasitic *R. culicivorax* was considerably lower than reported for most nematodes other than *Ascaris* (14). It is well established that small nematodes consume more O_2 on a weight basis than large ones (1,2,3,15,16) and direct comparisons are misleading (2). The lack of an effect of exogenous glucose on O_2 utilization and RQ probably indicates a lack of ability of the nematode to take up and utilize the sugar for metabolism. It is generally accepted that mermithids do not feed during postparasitic development, and Rutherford and Webster demonstrated that glucose uptake occurs only in the parasitic stage of *Mermis nigrescens* (11).

Postparasitic L_3 have a RQ of about 0.4. Low respiratory quotients are common in nematodes; e.g., *Aphelenchus avenae, Caenorhabditis* sp. (5), and *Panagrellus redivivus* (12). Such a low RQ could result from incomplete oxidation of catabolic reserves or a functional glyoxylate pathway (5). We have found that lipid is converted to glycogen in *R. culicivorax* under aerobic conditions which suggested the presence of the glyoxylate pathway (Imbriani and Platzer, unpublished).

Unlike the case with many other nematodes (2,3,15,16), oxygen consumption remained relatively constant during the development of *R. culicivorax* except in one experiment where the nematodes were maintained under conditions simulating

Table 2. Respiration of *Romanomermis culicivorax* postparasites developing under aerobic and anaerobic atmospheres.

Days postemergence	Atmosphere	Stage	Time*	Q_{O_2}†
2	Aerobic	L_3	0	0.916 A‡
8	Aerobic	L_3	0	0.898 A
	Anaerobic	L_3	0	1.340 B
			0.5	1.562 C
			1.0	1.795 C
			1.5	1.789 C
			2.0	1.918 C
			3.0	1.864 C
			3.5	2.128 C
15	Aerobic	Adults	0	1.114 AB
	Anaerobic	L_3	0	1.765 C
			0.5	1.856 C
			1.0	1.986 C
			2.0	1.972 C
			3.0	1.920 C
			3.5	1.875 C

*Time since removal from aerobic (air) or anaerobic (95% N_2-5% CO_2) atmospheres.
†Mean of three replicates in spring water equilibrated with 21% O_2, 1% CO_2, and 78% N_2 mixtures.
‡Values followed by different letters are significantly different ($P \leq 0.05$).

those encountered in our standard *in vivo* culture system (9). Under these conditions, the dissolved oxygen decreased by half and the oxygen consumption of the nematodes increased significantly. The RQ increased significantly in the adult nematodes 23 and 30 days postemergence. The high RQ, 0.7, indicated incomplete carbohydrate or lipid catabolism.

The respiration of postparasitic *R. culicivorax* was suppressed by low concentrations of cyanide indicating that an electron transport system of the a-a_3 type was functioning. It can not be stated unequivocally that cyanide sensitivity indicated typical mitochondrial electron transport, since cyanide sensitive respiration has been reported for various helminths which do not appear to have the classical a-a_3 cytochrome systems (15,16). It seems that the same electron transport mechanisms are utilized by both L_3 and adults since both are equally sensitive to cyanide. The ability to survive exposure to cyanide is an indication of the nematode's ability to live in the absence of O_2 as demonstrated in an earlier study (6). The ineffectiveness of NaN_3 and DNP was probably the result of lack of permeation of the inhibitors. Permeation is an important consideration when inhibitors show no effect on intact nematodes (4). There are several examples in the literature of cyanide sensitive respiration unaffected by NaN_3 or DNP except at very high concentrations (4, 18).

Physiological concentrations (0.03 to 5%) of CO_2 did not affect the respiration of *R. culicivorax*, but elevated concentrations ($\geq$ 10%) inhibited respiration. The reason for inhibition by high CO_2 concentrations was not clear but might be the result of a shift towards an acid pH. The concentration of CO_2 dissolved in water in equilibrium with 10% gaseous CO_2 could be about 140 mg $\cdot$ L^{-1} and is greater than that usually found in ponds and approaching the maximum reported in the literature (6). In summary, it seems that carbon dioxide was not an important modifier of respiration of *R. culicivorax* except at very high concentrations.

R. culicivorax behaves as an oxygen regulator (10) at O_2 concentrations less than 20% ($\cong$ air), with a critical tension between 2 and 5% O_2, and as a conformer at higher concentrations. This critical oxygen tension agrees well with calculations of size-limited diffusion based on the model developed by Atkinson (3). Diffusion would limit respiration in a nematode the size of *R. culicivorax* at an ambient O_2 concentration of about 3.0%; a value which is close to the experimentally determined critical oxygen tension. In previous experiments, development was not affected by reduction in O_2 unless the concentration was below 1% (6). This is well below the calculated value for size-limited diffusion and critical tension. It seems development and oviposition were not affected by whatever O_2 utilizing processes were reduced as O_2 concentrations fell below the diffusion imposed minimum for aerobic metabolism.

Atkinson has suggested that oxyconformity may be caused by hyperactivity induced by relatively high O_2 tensions (2,3). The increased respiration of *R. culicivorax* postparasites at O_2 concentrations above 20% may be the result of hyperactive movement. It is doubtful, however, that there is any adaptive significance to oxyconformity in *Romanomermis* for it occurs only at O_2 concentrations exceeding that of air.

As is the case with many other nematodes (2,3,15,16), oxygen consumption by *R. culicivorax* was elevated after prolonged exposure to anaerobic conditions. Atkinson suggested that development of an O_2 debt would be of little adaptive value to a nematode which is not normally exposed to fluctuating O_2 tensions (3). It seems improbable, therefore, that the stimulated postanerobic respiration in *R. culcivorax* resulted from oxidation of toxic anaerobic end products, since after postparasites penetrate into the anoxic pond substrate it is unlikely that O_2 is encountered again. A second explanation appears plausible. It was demonstrated that exposure to O_2 stimulated subsequent development under anaerobic conditions and suggested that the nematode would probably encounter an aerobic environment upon emergence from the host (6). We found that under aerobic conditions lipid was catabolized and carbohydrate stores increased, but in the absence of O_2 carbohydrate was catabolized (Imbriani and Platzer, unpublished). The in-

crease of carbohydrate in the presence of O_2 would be of value in later exploitation of the anoxic pond sediment. In light of these observations, it is suggested that the postanaerobic stimulation of respiration resulted from rapid utilization of lipids which were either completely oxidized or used for gluconeogenesis (or both). The "O_2 debt" of *R. culicivorax* may be the result of experimental design (exposing the nematodes to O_2 after prolonged anaerobiosis) and does not reflect natural conditions. The reasons for an increased O_2 uptake (when compared with postparasites utilizing lipid without exposure to anoxic conditions) were obscure. Perhaps the accumulation of reduced substrates created a steeper diffusion gradient and O_2 uptake was thus stimulated (15).

Von Brand emphasizes that to determine whether parasites lead an aerobic or anaerobic existence, it is necessary to know the O_2 availability in their environments, reactions to anoxia, and factors determining respiratory rates (15). A review of the limnological literature suggests that *R. culicivorax* may have access to O_2 immediately after emergence but encounters anaerobic conditions during later stages of development (6). The nematodes were capable of developing normally in anaerobic CO_2 containing atmospheres but development was stimulated by rather low concentrations of O_2. The experiments discussed in this paper indicated that O_2 was utilized when available and respiration was regulated at decreasing O_2 tensions until size-imposed diffusion limitations resulted in a decrease. Essentially all O_2 uptake is cyanide sensitive and probably resulted from a typical mitochondrial electron transport. It is suggested the nematode is well adapted for existence as a facultative anaerobe.

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