Respiratory Energy Losses in *Podophrya fixa* Müller in Relation to Temperature and Nutritional Status

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SUMMARY

Respiration in well-fed and starved *Podophrya fixa* was measured by means of Cartesian diver microrespirometry at 5 °C intervals between 10 °C and 30 °C. At 10 °C encystment occurred. The optimum temperature for respiration was 25 °C where a rate of $41.3 \times 10^{-6} \mu\text{l h}^{-1}$ per individual was recorded in well-fed animals. Mean cell size also reached a maximum at 25 °C in well-fed individuals. Both respiration rate and mean cell size decreased with starvation, as did the rate of metabolism. A table of daily respiratory energy losses in well-fed and starved *Podophrya* is presented. The results and their implications are discussed in relation to other protozoa.

INTRODUCTION

In recent years, interest in the energetics of protozoan species has grown, largely because so little is known of the role and energy relationships of small invertebrates such as protozoa, nematodes and rotifers, in the natural environment. Few complete energy budgets exist for protozoans, except for *Acanthamoeba* spp. (Heal, 1967) and *Stentor coeruleus* (Laybourn, 1976); but there have been many detailed investigations into what may be described as the feeding and growth energetics of protozoans, for example in *Tetrahymena pyriformis* (Curds & Cockburn, 1968, 1971), in *Entodinium caudatum* (Coleman, 1964) and *Colpidium campylum* (Laybourn & Stewart, 1975).

Very little data has been published on respiratory energy losses in protozoan species at normal environmental temperatures. The present investigation into respiratory energy losses in *Podophrya fixa* was undertaken to elucidate the temperature range of the species and to determine the effect of nutritional status on the rate of metabolism. *Podophrya* is a sedentary carnivorous suctorian species which produces motile ciliated 'larvae' by endogenous budding. When starved, reproduction ceases; but *Podophrya* can survive for many days without food and resume normal feeding and reproduction when the food supply is restored.

METHODS

Oxygen consumption. Oxygen consumption was determined in small stoppered Cartesian divers (Linderstrøm-Lang, 1943; Klekowski, 1971) of between 0.60 and 1.00 μl gas phase. The number of suctorians per diver was between 20 and 40, depending on the experimental temperature and nutritional state of the animals. The organisms were washed through five changes of sterile soil extract medium (Vickerman & Cox, 1967) to remove adhering bacteria. They were then drawn into a calibrated breaking pipette into which the length of air which constituted the gas phase of the diver had already been inserted. In this way both the organisms and the gas phase were introduced into the diver in one manipulation. Controls

were run on the final washing medium used in preparing the organisms in order to determine if any bacterial respiration was present.

Experiments were carried out at 10, 15, 20, 25 and 30 °C. At each temperature, oxygen consumption was determined in well-fed animals and animals starved for 48 h and 96 h. Five replicate experiments were conducted in each case.

An oxycaloric coefficient of 4.825 cal/ml oxygen (Phillipson, 1970) was used to convert values of oxygen consumed into units of energy. The SI unit of energy, the joule, is used in the present study (1 cal = 4.184 J).

Organism. *Podophrya fixa*, fed on *Colpidium*, was maintained in heavy watch-glasses filled with soil extract medium at the appropriate experimental temperature, so that the animals should be fully acclimatized. Animals which were to be starved were moved from the culture dishes to other dishes without food and incubated at the intended experimental temperature for either 48 or 96 h.

Determination of mean cell volume. *Podophrya fixa* is spherical and so cell volume may be calculated by employing the formula $\frac{4}{3}\pi r^3$. For each set of five replicate experiments, 50 animals from the same culture were measured using a high-power microscope with a calibrated graticule in the eyepiece. Cell weights were obtained by using a conversion factor for protozoan protoplasm derived from calorimetry studies (Laybourn, 1973).

RESULTS

The oxygen consumption per individual *Podophrya* in relation to temperature is shown in Fig. 1. At 10 °C they underwent encystment despite the presence of abundant food organisms. Attempts to measure respiration in the cysts proved unsuccessful; even when 60 cysts were contained in one diver, the changes in pressure recorded on the respirometer manometer were so small that no reliable result was obtained. Between 15 °C and 25 °C, oxygen consumption by well-fed animals rose to a maximum rate of $41.3 \times 10^{-6} \mu\text{l h}^{-1}$ per individual. Above 25 °C, oxygen consumption decreased sharply to a mean rate of $12.15 \times 10^{-6} \mu\text{l h}^{-1}$ per individual at 30 °C. Starved animals exhibited much lower respiration rates; when starved for 48 or 96 h, the peak rate of oxygen consumption occurred between 20 °C and 25 °C, thereafter declining gradually (Fig. 1). The magnitude of the acceleration of the respiratory process up to the optimum rate, expressed as Q_{10} , was 5.789 between 15 °C and 20 °C, and 3.754 between 20 °C and 25 °C in well-fed animals. In starved individuals Q_{10} between 15 °C and 20 °C was 2.567 after 48 h starvation and 2.888 after 96 h starvation.

The mean cell volume of *Podophrya* was also a function of temperature and nutritional state (Fig. 2). In well-fed individuals, the mean cell size was similar between 15 °C and 20 °C, and was between a mean of $47902 \mu\text{m}^3$ at 15 °C and $49512 \mu\text{m}^3$ at 20 °C. At 25 °C the mean cell volume showed a sharp increase to $61123 \mu\text{m}^3$, but above 25 °C it decreased. Cell size in starved animals progressively decreased between 15 °C and 30 °C, and their volumes were very much smaller with a maximum mean cell volume at 15 °C of $20893 \mu\text{m}^3$ after 48 h starvation and $16892 \mu\text{m}^3$ after 96 h starvation.

Podophrya depresses its metabolism during starvation, and the extent to which this occurred becomes clearer if oxygen uptake is expressed in terms of respiration rate per unit body or cell weight (Fig. 3). Only at 15 °C do starved, and consequently small, *Podophrya* have a higher rate of oxygen uptake per unit body weight than large well-fed individuals. At all other temperatures examined, the rate is similar or lower in small animals.

Daily respiratory energy losses in *Podophrya* are given in Table 1.

Fig. 1

Fig. 2

Fig. 1. Oxygen consumption in individual *Podophrya fixa*. Vertical lines indicate standard errors. ●, Well-fed animals; ○---○, animals starved for 48 h; ○—○, animals starved for 96 h.

Fig. 2. Mean cell volumes of *Podophrya fixa*. Vertical lines indicate standard errors. ●, Well-fed animals; ○---○, animals starved for 48 h; ○—○, animals starved for 96 h.

Fig. 3. Oxygen consumption of *Podophrya fixa* per unit body weight. ●, Well-fed animals; ○---○, animals starved for 48 h; ○—○, animals starved for 96 h.

Table 1. *Daily respiratory energy losses (R) in Podophrya fixa*

Temp. (°C)	Well-fed			Starved for 48 h			Starved for 96 h		
	Dry wt (ng)	R (nJ/24 h)	S.E.M.	Dry wt (ng)	R (nJ/24 h)	S.E.M.	Dry wt (ng)	R (nJ/24 h)	S.E.M.
15	8.14	4278	367	3.65	2387	308	2.87	1684	482
20	8.42	10308	757	3.19	3825	680	2.53	3709	842
25	10.39	19942	1669	2.16	2860	308	1.85	2995	308
30	3.96	5861	308	1.85	2030	482	1.43	1447	390

DISCUSSION

The only other known investigation of respiration in *Podophrya* was that undertaken by Hull (1961). He used Cartesian diver microrespirometry, and quotes oxygen consumption rates of 320 $\mu\text{l}/10$ min per individual at 28.5 °C (i.e. 1920 $\mu\text{l h}^{-1}$). Hull also measured the respiration rate of *Paramecium*, a prey organism in his study, and found rates of 800 $\mu\text{l}/10$ min per individual (i.e. 4800 $\mu\text{l h}^{-1}$). Such respiration rates are extremely high for protozoans. *Paramecium* has been widely studied and rates between 1.22×10^{-4} and 5.51×10^{-4} $\mu\text{l h}^{-1}$ per individual at temperatures ranging between 20 °C and 26 °C have been reported (Kalmus, 1928; Howland & Bernstein, 1931; Stewart & Pringle, 1961).

Compared with other small active protozoa, the *Podophrya* in the present study have a low rate of respiration. *Tetrahymena*, for example, which is similar in size to *Podophrya*, has a respiration rate of 1.5×10^{-4} to 7.8×10^{-4} $\mu\text{l h}^{-1}$ per individual at 26 °C (Løvlie, 1963). However, sedentary protozoa tend to have lower rates of respiration than more active protozoan species. *Stentor coeruleus*, a large sedentary species, has an hourly respiration rate of 1.5×10^{-4} to 3.0×10^{-4} μl per individual at 20 °C and 1.5×10^{-4} to 1.8×10^{-4} μl per individual at 15 °C in animals weighing 860 ng (Laybourn, 1975), while at 24 to 25 °C a rate of 0.43×10^{-4} μl per individual has been reported (Sarojini & Nagabhushanam, 1967).

Vorticella sp. has a respiration rate of 0.166×10^{-4} $\mu\text{l h}^{-1}$ per individual at 15 °C for animals weighing 5.1 ng and 0.098×10^{-4} $\mu\text{l h}^{-1}$ per individual at 20 °C for animals weighing 4.42 ng (Laybourn & Finlay, 1976). Thus the respiration rates obtained here compare closely with those reported for other sedentary protozoa, allowing for weight and optimum temperature differences.

The effect of temperature on the rate of oxygen uptake in protozoans has formed the basis of several studies. An optimum temperature for respiration of 25 °C has been found in *Spirostomum ambiguum* (Sarojini & Nagabhushanam, 1966) and in *Tetrahymena gelii* (Pace & Lyman, 1947). The optimum temperature for normal well-fed *Podophrya fixa* was also 25 °C. Other species, such as *Paramecium aurelia* and *Paramecium caudatum*, show a progressive rise in respiration rate between 15 °C and 35 °C (Pace & Kimura, 1944), so in these species the optimum temperature must be very much higher than in *Podophrya*, *Tetrahymena* and *Spirostomum*. The optimum temperature for respiration has ecological significance, since it must be one of the determining factors in the seasonal distribution and abundance of ciliate species.

Within a given species, smaller animals have higher respiration rates per unit body weight than larger individuals. Data available for the ciliate *Stentor coeruleus* (Laybourn, 1975), for example, show that individuals of 200 ng dry wt have an hourly respiration rate per ng that is 27 % higher than that of animals weighing 1000 ng. In starved *Podophrya*, the rate of oxygen uptake per unit weight was lower than in the larger well-fed individuals, which suggests that the rate of metabolism was greatly reduced during starvation. The changes in

metabolism and cell size induced by starvation have been considered in other protozoan species. *Paramecium caudatum* decreases its rate of oxygen uptake by 29 % after 12 h without food at 20 °C, and the reduced rate of respiration is accompanied by a progressive decrease in cell size (Leichsenring, 1925). In Podophrya, the greatest percentage decrease in respiration occurred at 25 °C. At this temperature the rate decreased by 84.9 % after 96 h without food, but the basic pattern manifested by starvation was similar to that in *P. caudatum*, with decreased respiration and reduced cell volume. *Chaos chaos*, a large species of carnivorous amoeba which usually dies about a month after the initiation of starvation, decreases its cell volume to about a quarter by the time death occurs (Holter & Zeuthen, 1947). Holter & Zeuthen also found that in *Chaos* the ratio between respiratory rate and reduced weight was constant and independent of the shrinkage of the amoebae. This suggests that in *Chaos* the rate of respiration per unit weight was constant throughout starvation, whereas in Podophrya the rate of metabolism per unit weight decreased as starvation progressed. Moreover, the reduction in the rate of metabolism was more pronounced at higher temperatures. The ability to reduce substantially its rate of metabolism, so as to conserve energy during periods of starvation, is advantageous to a sedentary organism such as *Podophrya* which is unable to swim and search for food.

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