AEROBIC METABOLISM AND PHYSIOLOGICAL RESPONSES OF AQUATIC OLIGOCHAETES TO ENVIRONMENTAL ANOXIA: HEAT DISSIPATION, OXYGEN CONSUMPTION, FEEDING, AND DEFECATION[1]

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(Accepted 4/21/87)

Anoxic heat dissipation of *Lumbriculus variegatus,* as measured by direct calorimetry, is reduced by up to 85% relative to aerobic rates. The decrease of anoxic heat dissipation and the diminution of activity peaks in the calorimetric output coincide with the disappearance of peristaltic movements under anoxia. A transfer from aerobic conditions with food to anoxia without food results in cessation of defecation when the gut is half emptied, whereas the gut is completely emptied under aerobic conditions within 6 and 8–10 h at 20 and 11 C, respectively. The aerobic retention time of the food is independent of worm length (10–50 mm). After aerobic feeding the gut content is higher than after anoxic feeding at 6 C. On return to aerobic conditions, heat dissipation increases immediately, whereas defecation is resumed only after a lag of 2 h. An anoxic component to the aerobic heat dissipation becomes apparent in relation to simultaneous respirometric measurements when feces accumulate in the calorimetric chamber. When the guts are completely emptied before the experiment, the theoretical oxycaloric equivalent yields an accurate estimate of heat dissipation, indicating that no significant net formation of anoxic end products occurs under aerobic conditions. Anoxic catabolism of glycogen may not fully explain the directly measured rates of heat dissipation under environmental anoxia. This has been suggested earlier for *Lumbriculus* and has since been confirmed for *Tubifex* on the basis of simultaneous calorimetric and biochemical measurements. Direct calorimetry is required to assess total rates of metabolic energy expenditure in anoxic oligochaetes.

INTRODUCTION

Oligochaetes were important subjects in the investigation of biochemical adaptations of invertebrates to environmental anoxia (Schöttler and Schroff 1976; Schöttler 1977, 1978; Gruner and Zebe 1978; Hoffmann 1981; Seuß, Hipp, and Hoffmann 1983; Putzer, Gnaiger, and Lackner 1985). These studies show that aquatic oligochaetes known to survive long periods of anoxia (Alsterberg 1922; Lindemann 1942; Brand 1946) utilize predominately the propionate-acetate pathway after short-term acclimation to anoxia. Excretion of propionate and acetate is common in euryoxic invertebrates (Hochachka and Somero 1984). This glycolytic pathway yields 6.3–6.4 mol adenosine triphosphate (ATP) per mole of glycosyl-unit in glycogen (Gnaiger 1977) instead of only 3 mol ATP/mol glycosyl-unit in the classic lactate pathway. Furthermore, as a result of the high ergodynamic (Gibbs energy) efficiency, the propionate-acetate pathway operates at a low rate (Gnaiger 1983*a,* 1987). The steady-state rate of heat dissipation of anoxic oligochaetes as measured by direct calorimetry is reduced by up to 80% relative to aerobic rates (Gnaiger 1979, 1980*a;* Famme and Knudsen 1984). This indicates a pronounced decline of steady-state energy requirements, which is an important adaptation to compensate for the lower ATP yield of anoxic relative to aerobic catabolism (maximally 6.4 vs. 37 ATP/glycosyl-unit).

Anoxic metabolism, however, is not restricted to anoxia but can be invoked under oxic (hypoxic or aerobic) conditions at low P_{O_2} and during intense locomotory activity. In fact, the involvement of anoxic pathways in routine metabolism even under air saturation is a controversial matter. We therefore investigated the aerobic routine me-

[1] Supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich, projects no. J0011 and J0187B.

Physiol. Zool. 60(6):659–677. 1987.

 0031-935X/87/6006-6105$02.00

tabolism of *Lumbriculus variegatus* by simultaneous direct calorimetry and respirometry and studied its metabolic responses to environmental anoxia.

A behavioral mechanism to restrain anoxic energy demands is the cessation of locomotory activity as shown by observation (Alsterberg 1922) and by direct calorimetry (Gnaiger 1980*a*, 1983*a*). In the natural environment, however, locomotory quiescence not only implies a diminished energy demand, but it simultaneously reduces the energy supply. Feeding and defecation are, therefore, important components of the physiological responses to anoxia in euryoxic invertebrates, especially in oligochaetes dwelling in reduced sediments of high organic content.

MATERIAL AND METHODS

ANIMALS

Oligochaetes were purchased from a local aquarium supply firm. *Lumbriculus variegatus* was sorted out and acclimated for at least 5 days in open-flow aquaria in a 12L:12D cycle. Supersaturation of the heated tap water had to be carefully avoided since *L. variegatus* is highly sensitive to gas bubble disease. The dry weight ($_dW$) of different batches of worms varied between 12% and 17% of the wet weight ($_wW$). A linear length-weight regression was obtained in the range of 10–55 mm length (r = .96; N = 26), with an intercept not different from zero. The constant of proportionality was 0.242 mg $_wW$/mm length (0.0288 mg $_dW$/mm length). The individual $_dW$ varied from 1.1 to 2.0 mg in the calorimetric experiments and ranged from 0.3 to 1.4 mg in the defecation experiments.

Homogenized and frozen spinach was washed, and the green water was decanted until it appeared clean. The spinach sediment provided the substrate for microbial growth and was used as food after an incubation period at room temperature of 4–10 days. It was added periodically (2–3 days) to the animals dwelling in washed sand (0.5-mm grain size), which appeared to provide a superior substrate relative to finer sand (0.06–0.1-mm grain size).

For calorimetric experiments with starved animals, *L. variegatus* was acclimated at 16 or 20 C under aerobic conditions for 3–20 days without addition of food, but microbial growth in the water tanks and consumption of dead specimens resulted in uncontrolled feeding conditions. Prior to experiments, the animals were cleaned and weighed. The fraction of $_dW$ of the $_wW$ was determined on separate animals or after experiments.

CALORIMETRY AND CALORESPIROMETRY

An LKB-2107 flow-sorption microcalorimeter was used as described by Gnaiger (1979, 1980*a*). It was equipped with gold capillary tubes to ensure anoxic perfusion of water equilibrated with pure nitrogen at experimental temperature. In another series of experiments (calorespirometry), a prototype Cyclobios Twin-Flow Respirometer (Gnaiger 1983*b*) was connected to the microcalorimeter, which enabled the continuous measurement of heat dissipation and oxygen uptake simultaneously. Groups of 10 animals were used at a perfusion rate of 4.17 mm^3/s (15 cm^3/h ± 0.7% SD). Aerobic (at air saturation) blank oxygen consumption (0.01–0.03 nmol O_2/s) and anaerobic blank oxygen diffusion (0.01 nmol O_2/s) were determined after each experiment and subtracted from the experimental rates (0.13–0.22 nmol O_2/s). Anoxic conditions are here defined as P_{O_2} < 0.07 kPa (< 0.5 mmHg) or < 1 µmol O_2/dm^3 (1 kPa = 0.133322 mmHg). Experimental conditions are referred to as *anaerobic* (without air) when the establishment of anoxic conditions was attempted but could not be achieved because of oxygen diffusion. To avoid this problem, the new Twin-Flow Respirometer is equipped with diffusion-free microvalves (Cyclobios 1985).

FEEDING AND DEFECATION

Aerobic and anoxic feeding experiments were performed in 10-cm high, 240-cm^3 glass jars sealed with Teflon-coated metal tops. Stainless steel capillaries and a Pasteur pipette were glued into bores for inflow and outflow and for the anoxic addition of food, respectively. The glass jars were filled up to 3–4 cm with sand, and 20 specimens were added. Water for perfusion of the animal chambers (45 cm^3/h) was equilibrated with air or pure nitrogen at 6 or 20 C. It was sucked with a peristaltic pump through the animal chambers, which were immersed in constant-temperature water baths. At 6 C

no mortality was observed after 40 days of aerobic or anoxic perfusion.

Defecation experiments were performed in July and August with animals fed in the late evening. In the morning of the next day, single individuals were pipetted from the sediment into glass vials for aerobic defecation, and into 10 × 10 mm-stoppered glass cuvettes for anoxic defecation. Anoxic perfusion was maintained with N_2-equilibrated water through stainless steel capillaries. Defecation was observed without food and sediment in constant-temperature rooms at 11 C (6 C acclimated) or at 20 C (20 C acclimated). Without disturbing the animals, we measured the length of the fecal pellets produced under the dissecting microscope by reference to a millimeter scale beneath the glass vial or cuvette. Readings were taken in 5–30-min intervals for up to 9–10 h. No reingestion of feces was observed. After experiments, the length of the live worms was measured using the same method as for the feces.

RESULTS

AEROBIC METABOLISM AS A REFERENCE STATE

Open-flow or perfusion instruments enable long-term monitoring of metabolic rate without depletion of oxygen. This is essential for establishing a normoxic reference state of metabolism, which in euryoxic organisms is considered as the aerobic state (i.e., close to air saturation; at 580 m above sea level or 95 kPa, 712 mmHg barometric pressure, it corresponds to P_{O_2} of ca. 19.4 kPa). The variability of the level and pattern of aerobic heat dissipation of starved *Lumbriculus variegatus* is shown in figure 1. Between all superimposed segments of the 6-day experiment, the animals were exposed to anoxia for 13–18 h. After an aerobic overshoot during recovery (see fig. 9), heat dissipation returned to preanoxic levels within the variability limits of the aerobic steady state. Unexpectedly, the final rate (sec. *IV*) was even higher than the initial rate (sec. *I*), despite the catabolic weight loss calculated at 9% of the starting ${}_dW$, owing to aerobic metabolism only (average aerobic rate −6.2 mW/g ${}_dW$, multiplied by 93 h of aerobic exposure and by 0.152 10^{-3} g catabolic weight loss per hour per milliwatt aerobic heat dissipation; Gnaiger [1983*c*]). On average, however, postanoxic steady-state rates (see fig. 9) were not significantly (1%–3%) less than preanoxic rates.

The irregular or occasionally regular (fig. 1, sec. *III*) patterns of heat dissipation reflect the activity fluctuations of the group of animals, since the same fluctuations were apparent in the traces of oxygen uptake (fig. 3*B*) but were absent in the calorimeter baseline (fig. 4). Moreover, the spontaneous activity peaks diminished under anoxia. However, the average increment of routine activity, that is, the difference between total routine rate and the relatively constant minimum level of heat dissipation (fig. 1), remained below a constant *fraction* of the total routine rate. Regardless of aerobic and anoxic conditions and of temperature, the average increment of routine activity amounted maximally to 10% of the total rate (fig. 2). Typically, aerobic steady-state rates were considerably less variable than those shown in figure 1 (e.g., see figs. 4, 9).

In contrast to the nearly constant rate in starved *L. variegatus,* aerobic heat dissipation decreased exponentially in animals that were fed before the experiment, and oxygen consumption showed an even more pronounced decline (fig. 3*A,* sec. *I*). The experimental calorimetric-respirometric (CR) ratio Δ_tQO_2 (total measured heat dissipation per mol oxygen consumed) was initially −460 kJ/mol O_2. Whereas this CR ratio agreed with the theoretical oxycaloric equivalent ranging from −445 to −478 kJ/mol O_2 for the complete oxidation of lipid, protein, and carbohydrate (Gnaiger 1983*c*), the significant trend of the CR ratio beyond −500 kJ/mol O_2 might be interpreted as an increasing contribution of anoxic processes to total heat dissipation (fig. 3*A,* sec. *I*). However, after removal of the fecal material that had accumulated in the animal chamber, the starved animals showed low and constant rates, and the CR ratio of −470 kJ/mol O_2 again did not indicate any anoxic net processes occurring under aerobic conditions (fig. 3*A,* sec. *II,* 3*B*). This suggests that the apparent anoxic component was not due to the metabolic activity of the animals but was most likely related to the gradual increase of anoxic microbial processes that depended on the accumulation of fecal particles.

In figure 3*B*, the oxygen consumption rate, $\dot{N}O_2$, was corrected for the transit time from the animal chamber in the microcalorimeter to the outflow polarographic oxygen sensor of the Twin-Flow Respirometer, but no corrections were made for the exponential time constants of the detector systems. The instrumental stability within an experiment is high (Gnaiger 1979, 1983*b*) such that the trend of the CR ratio from −470 to −500 kJ/mol O_2 within an experiment is significant (fig. 3*A*). However, difficulties in determination of the levels of blank oxygen consumption and of the calorimeter baseline in different experiments are responsible for the lower accuracy of absolute rates relative to the high resolution of changes within experiments. This is a major factor contributing to the index of variation in $\Delta_t QO_2$ of 8.4% (table 1). Different methods of baseline determination were used, and individual results of the measured CR ratio ($\Delta_t QO_2$) were not different from the theoretical oxycaloric equivalent, $\Delta_k HO_2$, in comparison to methodological accuracy. The measured CR ratio of starved aerobic *L. variegatus* averaged −450 kJ/mol O_2, in agreement with the theoretical oxycaloric equivalent for the catabolism of a mixture of lipid and protein (table 1). Therefore, anoxic metabolism was not involved under aerobic conditions.

TRANSITION TO ANOXIA

When *L. variegatus* was transferred to anoxic conditions after feeding, defecation stopped when the gut was 50%–60% empty (fig. 5). This occured after 2 h anoxia at 20 C (fig. 6). Similarly, metabolism declined immediately when oxygen was removed (fig. 4). After a period of 2–5 h, anaerobic

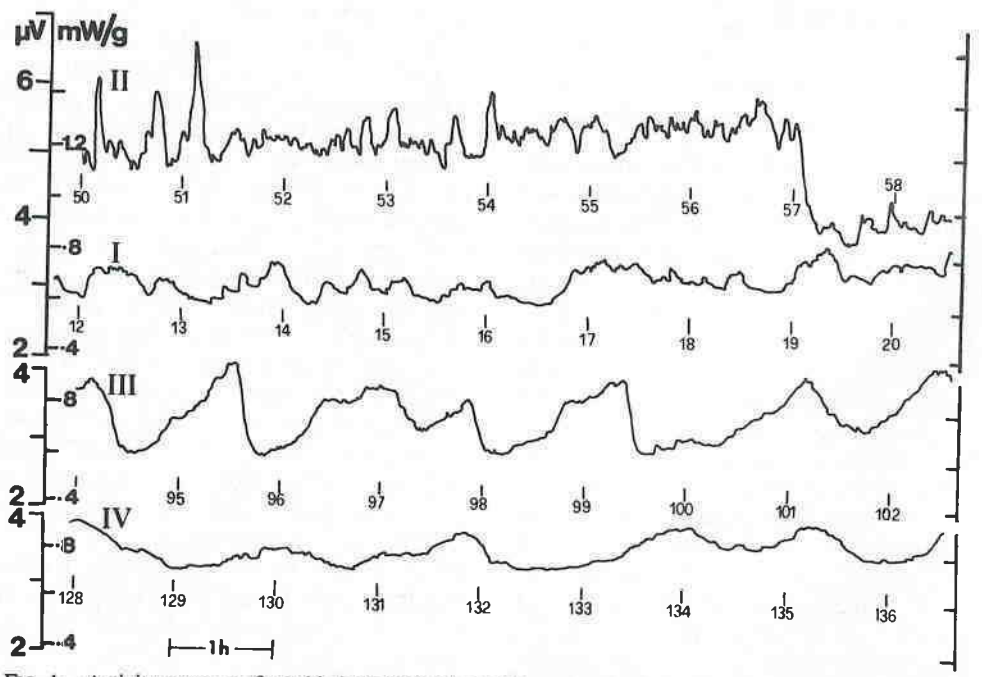

FIG. 1.—Activity patterns of aerobic heat dissipation of *Lumbriculus variegatus*, starved. Four superimposed segments of a calorimetric experiment are shown. The time (in hours) from the beginning of the experiment is indicated beneath each segment. The rate is given in mW/g $_wW$, and the corresponding voltage (1 µV = −18.2 µW) across the thermopiles is the recorded signal of the heat conduction calorimeter. The calorimeter baseline was at −0.2 µV. The total integrated and averaged rates of heat dissipation (given below in parentheses) are the "routine rates," and the relatively constant levels between periods of increased activity are the "level routine rates." 20 C; 1.7 mg $_dW$, 9.8 mg $_wW$ per individual, seven worms in the experimental chamber; perfusion 0.92 mm³/s. *I*, 0–27 h aerobic (12–20 h shown, −5.4 mW/g $_dW$), 27–45 h anoxic (−2.4 mW/g $_dW$); *II*, 45–62 h aerobic (50–58 h shown, −7.9 mW/g $_dW$), 62–75 h anoxic (−3.0 mW/g $_dW$); *III*, 75–103 h aerobic (94–102 h shown, −5.9 mW/g $_dW$), 103–118 h anoxic (−2.7 mW/g $_dW$); *IV*, 118–139 h aerobic (128–136 h shown, −5.9 mW/g $_dW$).

or anoxic rates remained constant for up to 48 h (Gnaiger 1980*a*). Under anoxia, fluctuations, which differed significantly from instabilities of the baseline, were observed only occasionally (figs. 2, 4). In the experiment shown in figure 4, anaerobic (hypoxic) heat dissipation amounted to 23% of the normoxic rate, but an oxidative contribution of about 6% of the normoxic rate was calculated from the measured diffusion of oxygen into the system (inflow P_{O_2} 0.00 kPa, outflow P_{O_2} 0.10 kPa with animals [fig. 4] and 0.27 kPa without animals).

Aerobic and anoxic defecation rates were measured as a function of animal size at two temperatures. Since we were interested in the problem of aerobic and anoxic peristaltic activity rather than in food-energy absorption per se, the length rather than the volume or mass of the feces had to be measured. The gut contents of fed animals, as quantified after aerobic defecation, were independent of feeding temperature (6 and 20 C), the added length of fecal pellets being nearly identical with the length of the worms (fig. 5). Under anoxia, however, defecation was incomplete at a retention of 40%–50% of the gut contents, independent of animal size and temperature (fig. 5).

Defecation velocity (mm feces s^{-1}) was a function of worm length such that the

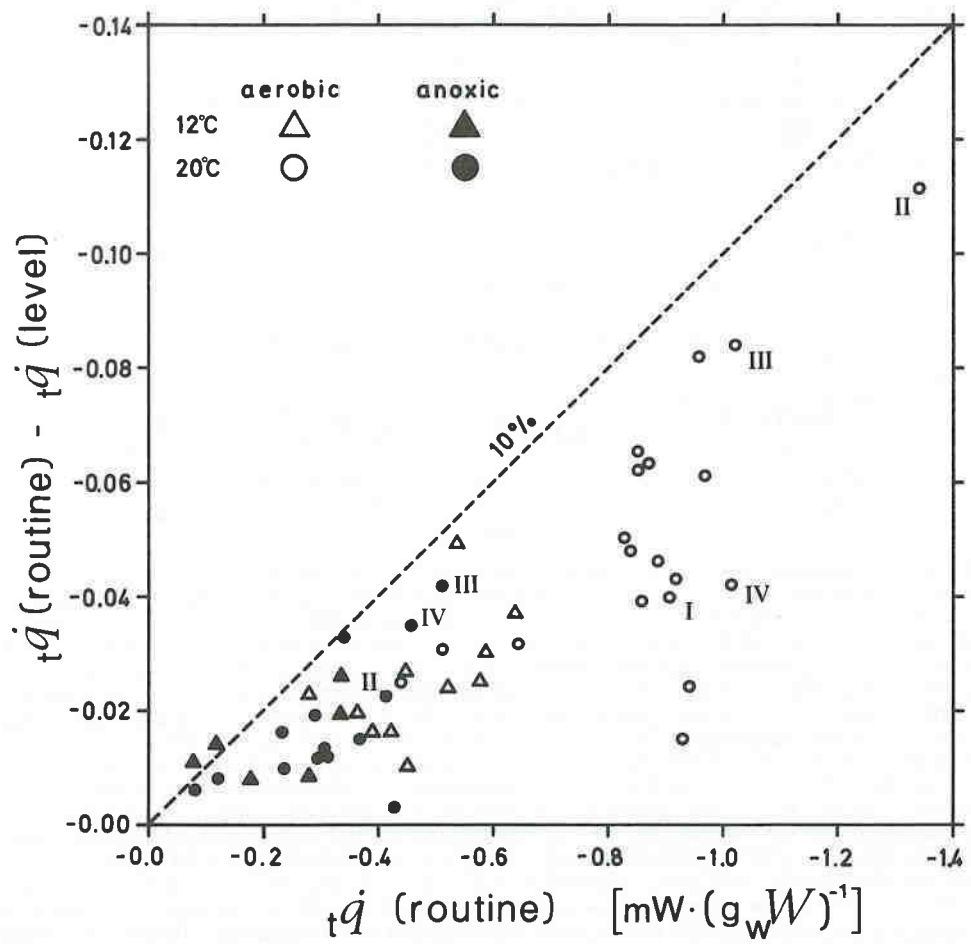

FIG. 2.—The "average increment of routine activity," $_t\dot{q}$(routine) − $_t\dot{q}$(level), in relation to the specific routine rate of heat dissipation, $_t\dot{q}$(routine), in *Lumbriculus variegatus* under aerobic and anoxic conditions and at two temperatures in 10 experiments. Each data point was obtained from experimental sections averaging 10 h during aerobic and anoxic steady states, excluding periods of aerobic recovery from anoxia (from Gnaiger 1983*a*). Roman numbers refer to the sections of the experiment shown in fig 1.

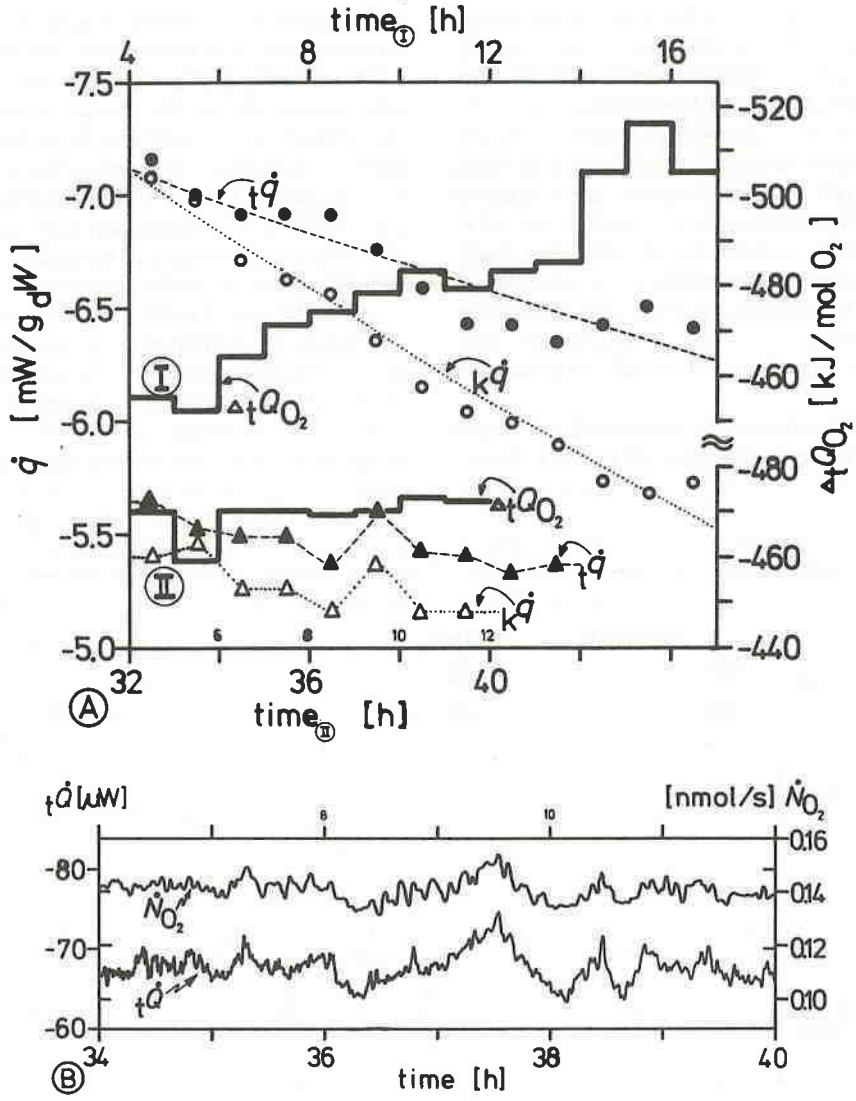

FIG. 3.—Simultaneous measurement of aerobic heat dissipation and oxygen consumption of *Lumbriculus variegatus* after feeding (*A*, sec. *I*), and after starvation and intermittent removal from the calorespirometer (*A*, sec. *II*; *B*). P_{O_2} = 19.3 and > 15.8 kPa (145 and >118 mmHg) in the inflow and outflow of the Twin-Flow respirometer, respectively. 20 C; 1.2 mg $_dW$ per individual, 10 worms in the experimental chamber; perfusion 4.14 mm^3/s. *A*, two superimposed segments of the experiment. Closed symbols: $_t\dot{q}$ (mW/g $_dW$), the total measured heat dissipation averaged over 1-h periods. Open symbols: $_k\dot{q}$, oxygen consumption converted into catabolic heat dissipation on the basis of the general oxycaloric equivalent, $\Delta_k H_{O_2} = -450$ kJ/mol O_2. Bars: $\Delta_t Q_{O_2}$ (kJ/mol O_2), the ratio of total heat dissipation and oxygen consumption, or CR ratio (note that this scale is not continuous through secs. *I* and *II*). *I*, 0–17 h after feeding (4–17 h shown). The exponential decrease of total heat dissipation (fitted stippled line) is 12% less than that of oxygen consumption (calculated catabolic heat dissipation, fitted dotted line). Therefore, the CR ratio increases, indicating a progressively important contribution of anoxic processes (see Discussion). At 17 h the animals were removed from the experimental chamber together with a large amount of fecal material. *II*, 28–40 h after beginning of experiment *I* (32–40 h shown). From 17–28 h the animals were starved in clean aerobic perfusion chambers for defecation of previously reingested fecal material and were replaced into the system at 28 h, after the baselines were determined (cf. fig. 5). The CR ratio was close to the initial value (*I*, 4–8 h) and did not change with time. *B*, chart recorder traces of heat dissipation, $_t\dot{Q}$ (µW), and oxygen consumption, $\dot{N}_{O_2}$ (nmol/s), of the section of the experiment shown in *A*, sec. *II*. Oxygen consumption is proportional to the P_{O_2} of outflow water (0.1 nmol O_2/s is equivalent to a change of P_{O_2} of 1.76 kPa = 13.2 mmHg).

TABLE 1

AEROBIC RATES OF TOTAL HEAT DISSIPATION, ${}_t\dot{q}$ ($mW \cdot g\ {}_dW^{-1}$), AND OF OXYGEN CONSUMPTION, $\dot{n}O_2$ ($nmol\ O_2 \cdot s^{-1} \cdot g\ {}_dW^{-1}$), OF *Lumbriculus variegatus* AT 20 C

Experiment	${}_t\dot{q}$ ($mW \cdot g^{-1}$)	$\dot{n}O_2$ ($nmol \cdot s^{-1} \cdot g^{-1}$)	$\Delta_t QO_2$ ($kJ \cdot mol^{-1}$)	${}_dW$ (mg)	Baseline[a]
February a	−4.08	8.67	−471	1.5	Amoquar
February b	−3.59	7.98	−449	1.5	Amoquar
June a	−8.51	20.53	−415	1.1	Amoquar
June b	−4.98	12.42	−401	1.4	Formaldehyde
June c	−4.64	9.27	−502	1.6	Formaldehyde
June d	−5.47	11.69	−469	1.2	Empty
$\bar{X}$	−5.21	11.76	−451	1.4	
SD	1.75	4.63	38	.2	

NOTE.—The ratio of these simultaneous calorespirometric measurements is the CR ratio, $\Delta_t QO_2$ (mJ/μmol or kJ/mol O_2). February experiments: ${}_dW$ was 17% ${}_wW$; with antibiotics (1 g penicillin G and 0.2 g streptomycin sulfate per dm^3). June experiments: ${}_dW$ was 12% ${}_wW$ (the major difference was due to a different weighing method where water was retained within the batch of worms); no antibiotics added.

[a] The baseline was determined by killing the animals with solutions of 0.1% Amoquar (Gnaiger 1979) or 2% formaldehyde, or by intermittently removing the animals and replacing the empty chamber into the calorimeter (figs. 3, 4).

retention time of the food was independent of gut length (fig. 6). The defecation rate relative to worm length [(mm feces/mm worm) s^{-1}] declined exponentially with decreasing gut contents (fig. 6). Under aerobic conditions at 20 C, 50% and 90% of the gut

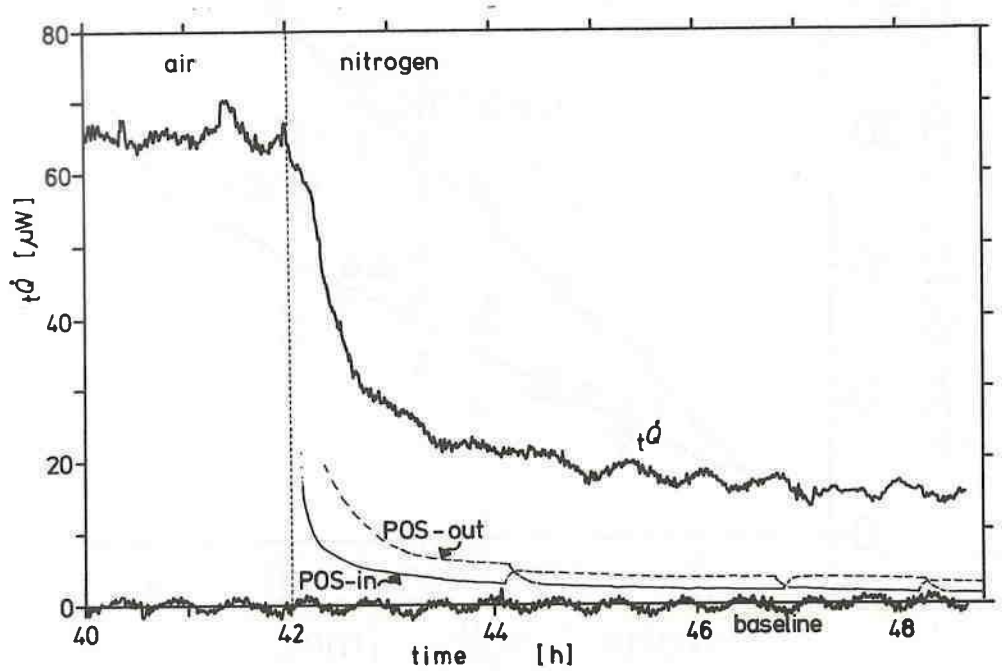

FIG. 4.—Aerobic-anaerobic transition of heat dissipation of *Lumbriculus variegatus* at 20 C (continuation of the experiment shown in fig. 3*B*; the baseline was determined in the empty system). The decline of ${}_t\dot{Q}$ follows closely that of oxygen as shown by the traces of the polarographic oxygen sensors in inflow (POS-in) and outflow position (POS-out). The Twin-Flow microvalves were switched after 44 h, before 47 h, and after 48 h. In the steady state, the higher outflow signals (0.10 kPa, 0.5% air saturation) after switching (stippled lines) relative to the zero inflow signals (solid lines) indicate diffusion of oxygen into the system.

was emptied after 2 and 5 h, respectively. During the first 3 h the aerobic egestion rate at 20 C was not higher than at 11 C, but the rate leveled off at the lower temperature after two-thirds of the gut was emptied (fig. 6). The anoxic defecation rate was lower than the aerobic rate at 11 C at all times. At 20 C the two rates were initially similar, but peristaltic arrest occurred within 2 h of anoxia (figs. 6, 8).

LONG-TERM ANOXIA AND AEROBIC RECOVERY

The lack of peristaltic activity under anoxia in the absence of food provides no indication that this physiologically important function is necessarily dependent on oxygen availability. To investigate this problem further, specimens of *L. variegatus* with completely empty guts were subjected to anoxic conditions in a perfusion system at 6 C. After 10 days' acclimation and continued starvation, food was added in the absence of oxygen (see Material and Methods). After 2 more days of anoxic feeding (12 days anoxia), four specimens were removed and defecation observed at 11 C under anoxic and aerobic conditions. The guts were 68% ± 7% SD full after anoxic feeding (fig. 7). The pattern of egestion had changed relative to aerobically acclimated specimens. Anoxic and aerobic defecation rates were identical and did not show the

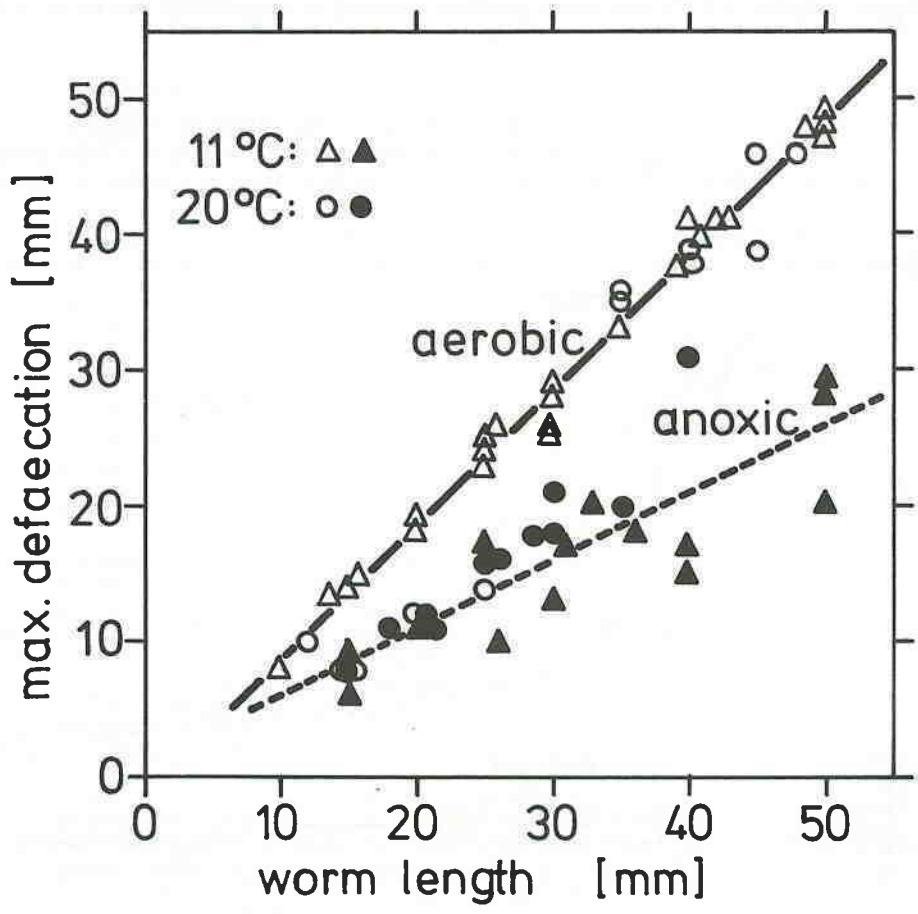

FIG. 5.—Maximum defecation (mm length of feces produced) after aerobic feeding as a function of length of *Lumbriculus variegatus* under aerobic and anoxic conditions during defecation, at 11 and 20 C. The slopes of the regressions were 0.99 (aerobic) and 0.49 (anoxic), indicating that 50% of ingested material was retained in the gut under long-term anoxia. The intercepts were not different from zero at $P < .05$. The results for specimens <25 mm at 20 C aerobic may be unreliable (because they were the first experiments before careful standardization) and were not included. 10–50 mm length corresponds to 2.4–12.1 mg $_{w}W$ (0.3–1.4 mg $_{d}W$).

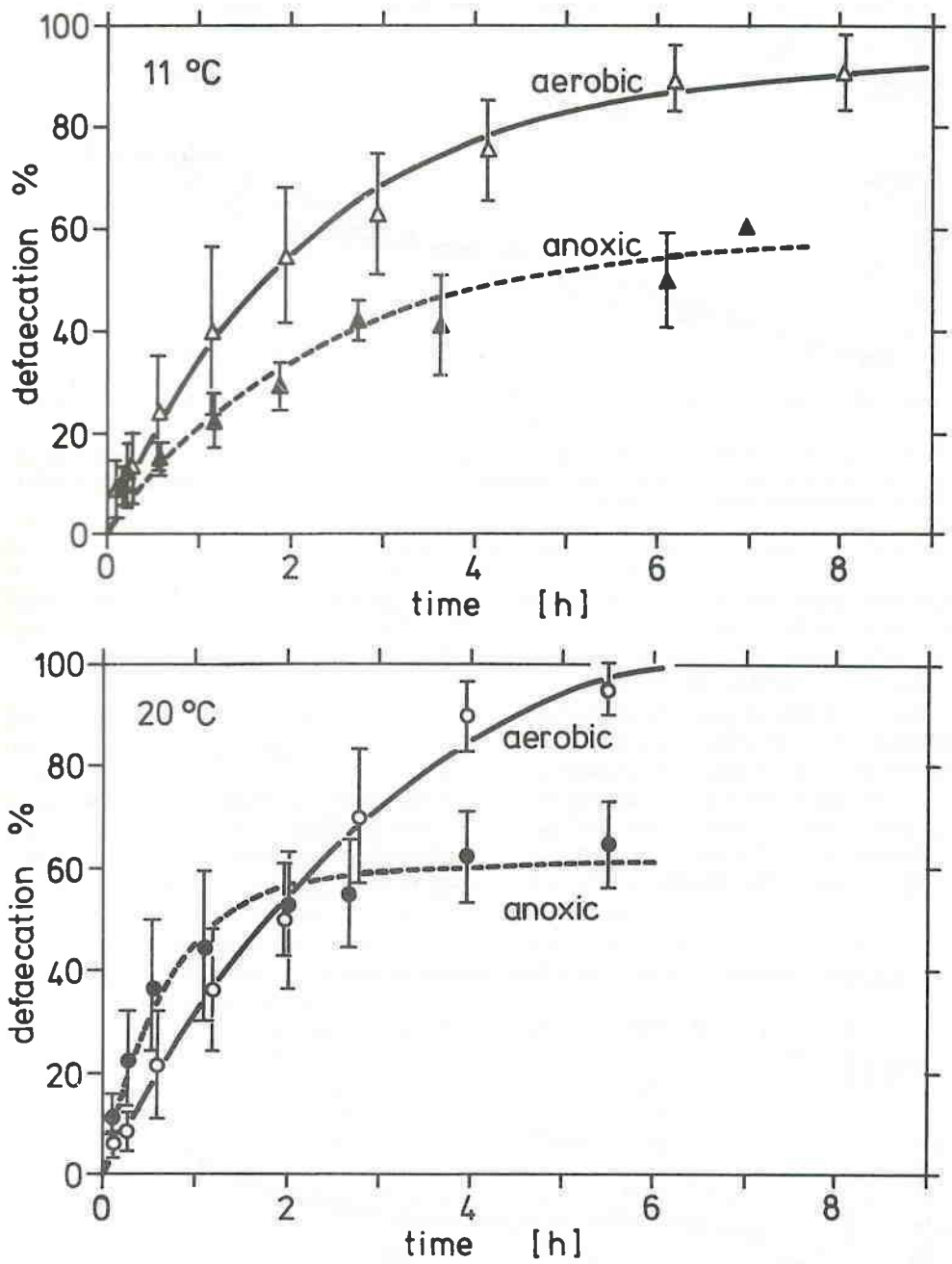

FIG. 6.—Time course of aerobic (*ox*) and anoxic defecation of *Lumbriculus variegatus*. Defecation is expressed as the cumulative length of feces in percentage of the length of the worms. The symbols and bars show the means ± SD, respectively, of grouped data. The regression lines were fitted according to the exponential equation

$$F = a(1 - e^{-bt})$$

where F and t are defecation and time, and a and b are parameters. In *A*, 11 C (after aerobic feeding at 6 C): aerobic, 10–50 mm length, 24 experiments, 224 data points; anoxic, 15–50 mm length, 14 experiments, 85 data points. In *B*, 20 C (after aerobic feeding at 20 C): aerobic, 25–50 mm length, 8 experiments, 98 data points; anoxic, 15–40 mm length, 11 experiments, 110 data points.

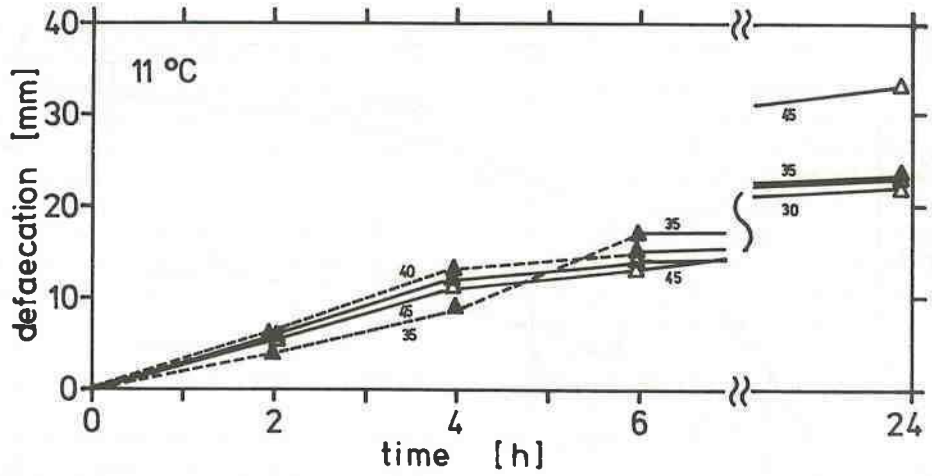

FIG. 7.—Time course of cumulative defecation (mm length of feces produced) at 11 C under aerobic (solid lines) and anoxic conditions (stippled lines), after anoxic feeding at 6 C. The numbers indicate the length (mm) of the four specimens of *Lumbriculus variegatus*.

exponential time course within the first 4 h after removal from the food.

Peristaltic arrest during short-term anoxia was fully reversible (fig. 8). After a lag period of 2 h of aerobic recovery, defecation continued normally. This lag period was not correlated with a similarly gradual increase of metabolic activity after anoxia. On the contrary, an overshoot of heat dissipation immediately followed the anoxic-aerobic transition (fig. 9). The postanoxic steady-state rate was typically obtained after 4 h of aerobic recovery (fig. 9; Gnaiger 1983*a*).

DISCUSSION

AEROBIC ENERGETICS

Respiration in aquatic oligochaetes, particularly of *Tubifex tubifex*, has been investigated using various methods. Manometric respirometry (Warburg, Gilson)

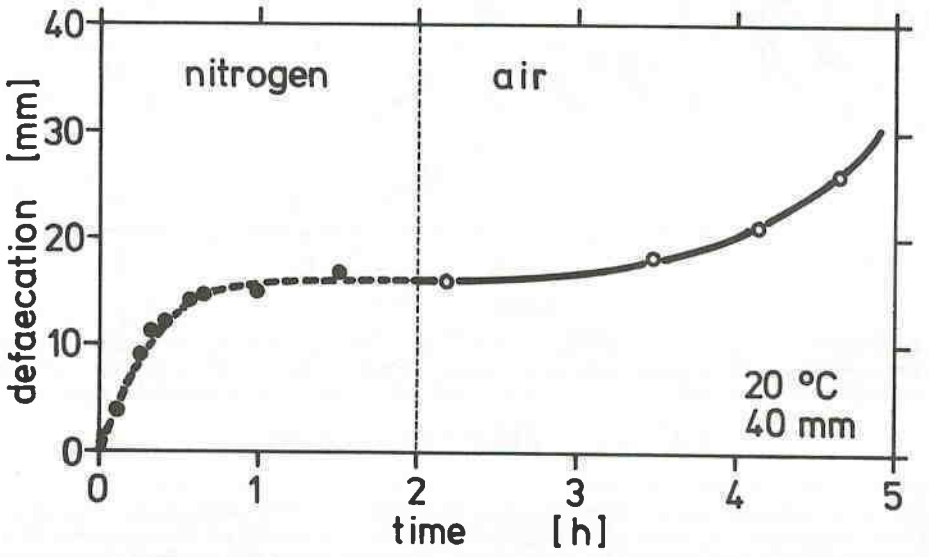

FIG. 8.—Cessation of defecation (mm length of feces produced after aerobic feeding) under anoxia (*anox*), and continuation of defecation after aerobic recovery (*ox*) of *Lumbriculus variegatus* (40 mm length) at 20 C.

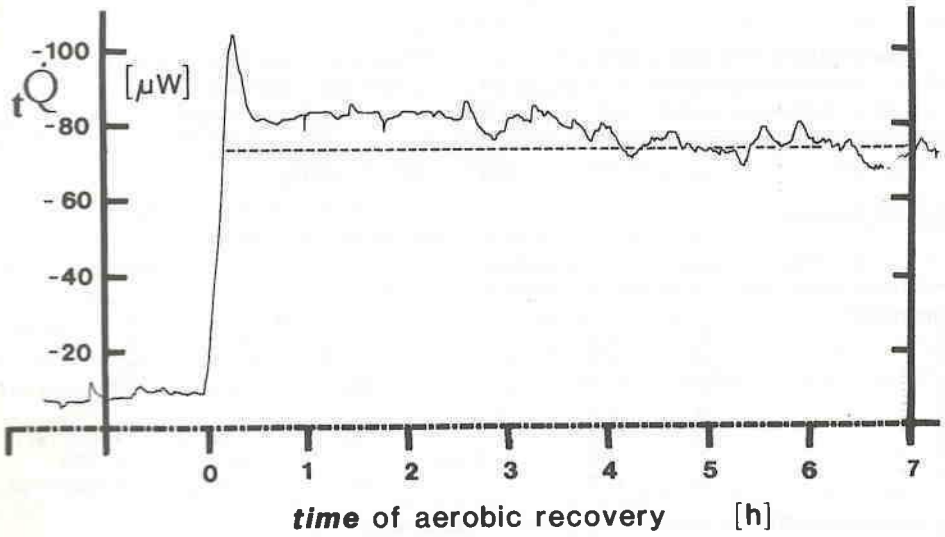

FIG. 9.—Heat dissipation of *Lumbriculus variegatus* during aerobic recovery from 17 h of anoxia. The stippled line shows the aerobic steady-state rate (−4.24 mW/g $_dW$) averaged over 4–10 h after the anoxic-aerobic transition (40–46 h of the experiment). The initial aerobic rate (4–18 h of the experiment) was −4.66 mW/g. Starved; 20 C; 1.7 mg $_dW$ per individual, 10 worms in the experimental chamber; perfusion 4.17 mm^3/s.

involving shaking of the animal chamber results on average in a twofold increase of oxygen uptake (data compiled by Gnaiger [1983*b*]; there are printing errors in the equations [p. 137], but tabulated values are correct). Only respirometers without high turbulence or vibrational disturbance of the oligochaetes yield reliable rate estimates, and these are referred to exclusively here. It is not possible at present to attribute the remaining variability (table 2) to either methodological differences (open-flow vs. closed systems, water current) or to specified physiological states of the animals. The coefficients to standardize for weight and temperature are approximations (table 2). Aerobic rates were unaffected by perfusion of 3.3 and 15 cm^3/h (table 2; Gnaiger [1980*a*] and this study, starved, respectively). The same results are obtained with groups of oligochaetes in the absence and presence of sediment (Brinkhurst, Chua, and Kaushik 1983). Within any particular study, the coefficient of variation typically ranges from ±5% to ±20% of the mean rate, but higher variabilities may be due to seasonal effects.

Interspecific differences of respiration in Tubificidae and Lumbriculidae are relatively small (table 2; Brinkhurst et al. 1983; Van Hoven 1975). Tubificids in combined culture exhibit a reduction in respiration rate of 40%, possibly by reducing locomotory activity (Chua and Brinkhurst 1973). Seuß et al. (1983) reported an aerobic rate of 5.3 nmol O_2 s^{-1} g $_dW^{-1}$ at 13 C (9.2 nmol s^{-1} g^{-1} normalized to 20 C) for a mixture of tubificids exposed to antibiotics. Depending on the type and concentration of antibiotics, metabolic rates of *Lumbriculus variegatus* decrease or increase significantly (Gnaiger 1983*d*), comparable to responses to sublethal concentrations of toxicological agents (Chapman, Farrell, and Brinkhurst 1982).

Aerobic activity may change considerably within an experiment as a function of time (fig. 1). In closed respirometers it is difficult to separate the effect of gradually declining P_{O_2} from that of time. In the attempt toward a standardized respirometric method, the Cyclobios Twin-Flow Respirometer was developed and applied in studies of aquatic animals (Gnaiger 1983*b*). Aerobic steady-state rates of *starved L. variegatus* stabilized within <4 h of the experiment, usually within the time of instrumental equilibration (1–3 h for the calorimeter). The rate for previously *fed* oligochaetes, however, declined exponentially

TABLE 2

COMPARISON OF PUBLISHED RESPIRATORY RATES OF *Tubifex tubifex* AND *Lumbriculus variegatus* AT EXPERIMENTAL TEMPERATURE, $\dot{n}O_2$(exp), TEMPERATURE CORRECTED FOR 20 C,[a] AND WEIGHT CORRECTED FOR 1 mg $_dW^b$ [1 ml O_2 h^{-1} = 12.393 nmol O_2 s^{-1}]

Species and Temperature (°C)	$_dW$ (mg)	$\dot{n}O_2$(exp) (nmol $O_2 \cdot s^{-1} \cdot g\ _dW^{-1}$)	$\dot{n}O_2$(20) (nmol $O_2 \cdot s^{-1} \cdot g\ _dW^{-1}$)	$\dot{n}O_2$(20; 1 mg) (nmol $O_2 \cdot s^{-1} \cdot g\ _dW^{-1}$)	Reference
Tubifex tubifex:					
19	?	11.6	12.5	...	Dausend 1931
14	1.0	9.1	14.6	14.6	Berg et al. 1962
20	1.0[c]	11.9	11.9	11.9	Johnson and Brinkhurst 1971
20	.4	6.4	6.4	5.1	Brinkhurst et al. 1972
15	.3	7.2	10.7	7.9	Chua and Brinkhurst 1973
10	?	4.8	10.6	...	Brinkhurst et al. 1983
15	.5	11.8	17.5	14.7	Famme and Knudsen 1984
Lumbriculus variegatus:					
20	1.7	11.3	11.3	12.9	Gnaiger 1980*a*[d]
20	1.2	13.9	13.9	14.6	Fed and feces (fig. 3*A*, *I*)
20	1.2	11.7	11.7	12.3	Starved (fig. 3*A*, *II*)
20	1.4	11.8	11.8	12.6	Starved (table 1)

NOTE.—For information on conversion factors for units of oxygen consumption, see Gnaiger (1983*c*).
[a] $\dot{n}O_2(20) = \dot{n}O_2(\text{exp}) \times Q_{10}^{(20-\text{Temp.})/10}$; $Q_{10} = 2.2$.
[b] $\dot{n}O_2(20; 1\text{ mg}) = \dot{n}O_2(20) \times {}_dW^{1-b}$; $b = 0.75$.
[c] Assuming 10% ash content.
[d] Calculated from heat dissipation.

by 25%–40% over 15 h in *T. tubifex* (Famme and Knudsen 1984) and in *L. variegatus* (fig. 3*A*, sec. *I*).

The bioenergetic interpretation of oxygen consumption of euryoxic invertebrates remains controversial. It was suggested that deep tissues of the bivalve *Modiolus demissus* are insufficiently oxygenated under aerobic conditions and function anoxically (Booth and Mangum 1978). Even then, net anoxic metabolism is not necessarily implied, since organic end products of anoxic cells may not be excreted but, instead, could serve as a substrate for aerobic peripheral cells and tissues (for vertebrate tissues, see Baumgärtl and Lübbers [1983]). Effective uptake of dissolved amino acids, rather than net excretion, is documented in euryoxic bivalves using sensitive methods (Manahan et al. 1982). Indeed, even during air exposure of *M. demissus,* time-averaged direct and indirect calorimetry agree (Pamatmat 1983; table 3). Simultaneous calorimetry and respirometry provide a direct method for the bioenergetic interpretation of oxygen uptake. However, nonsimultaneous measurements may involve different states of activity of the animals in the calorimetric and respirometric chambers. Not surprisingly, therefore, the variability of the CR ratio of nonsimultaneous measurements is high at a range of −320 to −510 kJ/mol O_2, but the mean is not significantly different from the aerobic oxycaloric equivalent (table 3, P). In contrast, simultaneous calorespirometric measurements of aerobic steady-state metabolism of euryoxic and stenoxic animals yield less variable CR ratios; the observed range of $\Delta_t Q O_2$ from −430 to −480 kJ/mol O_2 (table 3, S) is in line with the oxycaloric equivalent for aerobic dissipative catabolism, $\Delta_k H O_2$ (Gnaiger 1983*c*).

Such agreement, however, is limited to aerobic steady states. Glycolytic end products accumulate and are excreted below critical P_{O_2} levels (Livingstone and Bayne 1977; Schöttler, Wienhausen, and Zebe 1983; Pörtner, Heisler, and Grieshaber 1985). Therefore, anoxic and aerobic heat dissipation co-occur at low oxygen availability (Famme et al. 1981; Gnaiger 1983*b;* Hammen 1983; Pamatmat 1978; Shick 1981; Shick, DeZwaan, and De Bont 1983; Shick et al. 1986; Widdows and Shick 1985), whereas conservation of heat with

apparent efficiencies as high as 45% is observed during the anoxic-aerobic transition and recovery metabolism (Meyerhof 1920; Gnaiger, Shick, and Widdows, in preparation; Shick et al. 1986). In addition, any environmental or physiological stress may invoke anoxic short-term responses and hence cause significant differences between calorimetric and respirometric methods (Gnaiger 1981, 1983*a*, 1983*d*).

In early studies of aerobic steady-state respiration of *T. tubifex*, respiratory quotients, RQ (mol CO_2/mol O_2), of 1.32 and 0.70 are reported (Dausend 1931 and Harnisch 1935, respectively—the latter using the Warburg method). Koenen (1951) criticized the method of CO_2 analysis used by Dausend and confirmed the lower aerobic RQ values in *T. tubifex*. A result of RQ > 1 indicates the contribution of net anoxic processes to total aerobic metabolism except under conditions of carbohydrate to lipid conversion, but RQ = 0.7 corresponds to purely aerobic catabolism of lipid (Kleiber 1961). The calorespirometric results on *L. variegatus* (table 1) and on *T. tubifex* (Famme and Knudsen 1984; table 3) suggest the equivalence of direct and indirect

TABLE 3

CALORIMETRIC-RESPIROMETRIC RATIOS, $\Delta_t Q_{O_2}$ (kJ/mol O_2), AS MEASURED BY HEAT CONDUCTION CALORIMETRY AND VARIOUS RESPIROMETRIC METHODS IN POIKILOTHERMIC ANIMALS UNDER AEROBIC CONDITIONS

Taxon and Species	$\Delta_t Q_{O_2}$	Temperature	Reference
S Annelida:			
Lumbriculus variegatus	−450	20 w	Table 1
Tubifex tubifex	−480	15 w	Famme and Knudsen 1984
Neanthes virens	−460	15 w	Lowe 1978
Mollusca:			
Modiolus demissus	−470	20 a	Pamatmat 1983
Mytilus edulis	−470	20 w	Famme et al. 1981
Mytilus edulis	−450	15 w	Gnaiger et al., in preparation
Crustacea:			
Cyclops abyssorum[a]	−460	6 w	Gnaiger 1983*b*
Austropotamobius fluviatilis	−460	15 w	Lowe 1978
Uca pugnax	−430	27 a	Pamatmat 1978
Pisces:			
Gasterosteus aculeatus	−450	15 w	Lowe 1978
Salvelinus alpinus larvae	−450	6 w	Gnaiger 1983*b*
S $\bar{X}$	−460 ± 13 SD (± 3%)		
P Coelenterata:			
Actinia equina[b]	−410	15 a	Shick 1981
Mollusca:			
Mytilus edulis	−480	15 w	Gnaiger et al., in preparation
Biomphalaria glabrata	−380	. . . a	Becker and Lamprecht 1977
B. glabrata infected	−420	. . . a	Becker and Lamprecht 1977
Insecta:			
Formica polytena ants	−460	20 a	Coenen-Stass, Schaarschmidt, and Lamprecht 1980
F. polytena ants	−320	30 a	Coenen-Stass et al. 1980
F. polytena pupae	−450	20 a	Coenen-Stass et al. 1980
F. polytena pupae	−420	30 a	Coenen-Stass et al. 1980
Tenebrio molitor	−480	25 a	Peakin 1973
Tribolium confusum eggs	−340	30 a	Dunkel et al. 1979
T. confusum insects	−510	30 a	Dunkel et al. 1979
P $\bar{X}$	−420 ± 60 SD (± 14%)		

NOTE.—S = oxygen uptake determined in simultaneous calorespirometry; the range of experimental CR ratios agrees with the theoretical oxycaloric equivalent for aerobic dissipative metabolism. P = oxygen uptake determined in parallel experiments. a and w = animals in air or in water, respectively. Temperature = experimental temperature (C).

[a] Calculated for the routine activity component.

[b] Intertidally acclimated group.

calorimetry. However, Famme and Knudsen (1984) postulate the simultaneous importance of aerobic and glycolytic net rates in aerobic *T. tubifex,* reporting aerobic acetate and propionate production. This phenomenon may require a different interpretation on the basis of our present study: (1) After aerobic feeding, *L. variegatus* emptied its gut within 6 h at 20 C (fig. 6). The "animals were allowed to stay in tap water to clear their guts for 60 min before they were placed into the calorimeter ampoule" (Famme and Knudsen 1984; 15 C); then, about 50% of the gut contents (fig. 6) must have accumulated as feces in their calorimeter chamber. Approximately 30% of the bacteria passing through the gut of oligochaetes survive intact (Wavre and Brinkhurst 1971). Bacterial oxygen uptake in a respirometer increases steeply after 6 h of contact with animals even without contamination by feces (Dalla Via 1983). It is yet to be shown by the use of oxygen microelectrodes whether fecal pellets can provide a microenvironment for anoxic microbial activity (Revsbech 1983; Sexstone et al. 1985). (2) An anoxic component of total heat dissipation increased gradually after 8 h in our calorespirometric chamber contaminated by fecal pellets, combined with decreasing but high rates of heat dissipation and oxygen consumption (fig. 3*A*, sec. *I*). Since the rates decreased further and the CR ratio returned to the theoretical value after removal of the feces from the chamber (fig. 3*A*, sec. *II*), we have to conclude that the excess aerobic and the anoxic component of ${}_t\dot{Q}$ were due to microbial activity associated with the feces. (3) The steady-state respiration rate measured by Famme and Knudsen (1984) is among the highest reported for *T. tubifex* (table 2). The high apparent rate might be due to contamination by feces, although these authors do not address the methodological problem of microbial oxygen consumption in their system.

Evolutionary energetics introduces the question of the fitness gained by maintaining net anoxic catabolism under aerobic conditions. The continued accumulation of acidic (lactate, succinate, propionate, acetate) or toxic (ethanol) end products is intolerable for any known organism. Therefore, the only possible anoxic long-term mechanism is excretion of anoxic e products, which entails an excretory loss addition to the catabolic heat loss. The c responding substrate demand would rise 63% relative to fully aerobic glycogen cc sumption, if only 5% of total heat dissip tion were anoxic ($\Delta_t Q O_2 = -500$ instead -478 kJ/mol O_2). This calculation is bas on the assumption that propionate and a etate are excreted.

The aerobic enthalpy of combustion bomb calorimetric value, expressed as t *oxyenthalpic* equivalent, $\Delta_c H O_2$, is -4 kJ/mol O_2 for the combustion of glycog (Gnaiger 1983*c*). The enthalpy of combu tion of aerobic, plus 5% anoxic, glycog utilization is then -770 instead of -4 kJ/mol O_2 (calculated from data in Gnaig [1983*a*] and Gnaiger and Bitterlich [1984 For every mW of metabolic heat dissip tion, 1.5 mJ s^{-1} (instead of 1 mJ s^{-1}) substrate-combustion enthalpy would lost. Concomitant with the 5% anoxic he change, ATP is produced, and, of the to ATP turnover, 9% is generated anoxicall Expressed in terms of the carbohydrate a similation required to compensate for t catabolic input to produce 1 mol AT -111 kJ instead of -77 kJ combustion e ergy per mol ATP turnover are required 5% of the heat is anoxic compared to ful aerobic catabolism of glycogen. Therefo as little as 5% anoxic heat dissipation wou increase the cost of ATP production 1 44%. It is difficult to conceive how such poor metabolic strategy could be selecte for in euryoxic animals.

These theoretical considerations a supported by the observation of uptal rather than excretion of volatile fatty aci under aerobic conditions in *T. tubif* (Hipp, Sedlmeier, and Hoffman 198 Putzer et al. 1985) and in the polychae *Arenicola marina* (Holst and Zebe 1984)

ANOXIC ENERGETICS

Survival under anoxia depends on a su cessful compromise between a sufficient high ATP production rate to meet tl maintenance demands and an overall r duction of metabolic rate to save energ Tubificids, including *Tubifex templeto* and *Limnodrilus hoffmeisteri,* avoid anox and hypoxic conditions in an oxygen gr dient (Van Hoven 1975). In the aerotact

response of *L. hoffmeisteri* the "movement toward aeration was slightly more important than avoidance of hypoxia" (Fisher and Beeton 1975). Famme and Knudsen (1985*a*) were unaware of these studies and reported an apparent preference by *Tubifex* sp. for anoxic water. This is surprising since *T. tubifex* exhibits a "relatively low tolerance of anaerobic conditions" compared with *L. hoffmeisteri* (50% mortality at 20 C after 28.1 days and 52 days, respectively; Birtwell and Arthur [1980]; see also Chapman et al. [1982]). Dausend (1931) obtained 50% mortality of *T. tubifex* after only 3.5 days' anoxia at 20 C, as compared with 7 days for *L. variegatus* at the same temperature and starvation (Putzer 1985; see also Alsterberg 1922, p. 31).

Metabolic calorimetry is a noninvasive method for the measurement of aerobic and anoxic heat dissipation. Heat dissipation is a negative component, R, in the physiological energy budget where the energy equivalent of organic matter is based on bomb calorimetry. In aerobic catabolism of glycogen or lipid, the catabolic heat effect under cellular conditions (oxycaloric equivalent) and the bomb calorimetric heat effect under reference conditions for combustion (oxyenthalpic equivalent) are nearly equal (Gnaiger 1983*c*). However, in aerobic catabolism of protein and excretion of ammonia or urea, U, the oxycaloric (R) and oxyenthalpic ($R + U$) equivalents are different, −451 and −527 kJ/mol O_2, respectively (Gnaiger 1983*c*); aerobic heat dissipation must then be multiplied by 1.17 to obtain the combustion enthalpy, $R + U$, of which 86% is due to cellular heat dissipation, R. Propionate and acetate are the only quantitatively important glycolytic end products in tubificids after anoxic acclimation for 4–12 h (Seuß et al. 1983; Putzer et al. 1985). Excretion of these organic acids represents an energy loss, U, of −2,614 kJ/mol glycosyl-unit catabolized, concomitant with a catabolic heat loss, R, of −226 kJ/mol glycosyl-unit (Gnaiger 1983*a*). The sum, $R + U$, is the bomb calorimetric enthalpy of combustion, −2,840 kJ/mol glycosyl-unit. The catabolic heat dissipation in this anoxic pathway represents only 8% of the combustion enthalpy of the substrate glycogen. Therefore, anoxic heat dissipation associated with the propionate-acetate pathway has to be multiplied by 12.6 to obtain $R + U$. Minimum anoxic (anaerobic) steady-state heat dissipation of *L. variegatus* and *T. tubifex* is 20% of the aerobic rate (Gnaiger 1980*a*; Famme and Knudsen 1984) or even lower (fig. 4: 16% anoxic when the contribution of oxygen uptake is subtracted). Anoxic heat dissipation reduced to 16% of the aerobic rate then indicates an up to twofold loss to the energy budget under anoxia—172% (= 16% × 12.6/1.17) to 202% (= 16% × 12.6/1.0) relative to the aerobic $R + U$. Consequently, the reduction of metabolism by aquatic oligochaetes under anoxia does not fully compensate for the increased substrate-enthalpy cost of anoxic ATP production.

From 6.33 to 6.43 mol ATP are produced per mol glycosyl-unit in the propionate-acetate pathway (Gnaiger 1983*a*); that is a 5.8-fold cost of combustion energy per mol ATP turnover compared to aerobic catabolism (37 ATP/glycosyl-unit). The catabolic heat dissipation per mol ATP turnover, on the contrary, is lower in anoxic than in aerobic catabolism. For aerobic glycogen consumption the caloric equivalent of ATP turnover, $\Delta_k H\infty\mathrm{ATP}$, is calculated as

$$\Delta_k H\infty\mathrm{ATP} = \Delta_k H\mathrm{O}_2 \times (6.17\ \mathrm{mol\ ATP/mol\ O_2})^{-1} = -78\ \mathrm{kJ/mol}\ \infty\mathrm{ATP}.$$

When the volatile fatty acids are excreted in anoxic catabolism, the caloric equivalent of ATP turnover amounts to −36 kJ/mol ∞ATP, compared to −80 kJ/mol ∞ATP in aerobic catabolism of mixed substrates (Gnaiger 1983*a*). This reduction of the anoxic heat effect per unit ATP turnover to <50% of the aerobic value is important for the metabolic interpretation of calorimetry. Catabolic heat changes in propionate-acetate production and excretion have to be multiplied by 2.2 for comparison of relative rates of anoxic and aerobic ATP turnover. Anoxic heat dissipation of 16% would indicate an anoxic ATP turnover of 35% with respect to the aerobic rate. Anoxic ATP turnover of 27% of the aerobic rate was calculated from biochemical data of Seuß et al. (1983) for tubificids during 18 h anoxia,

using stoichiometric ratios of ATP and glycolytic end products (Gnaiger 1977, 1983*a*).

However, these calculations rest on the assumption that metabolic heat changes measured under anoxia agree with biochemical enthalpy changes (indirect calorimetry), an assumption comparable with the explanation of aerobic heat dissipation by the caloric equivalent of oxygen consumption (tables 1, 3). In anoxic oligochaetes this may not be the case, as was suggested on the basis of calorimetric measurements under hypoxia compared with published (Schöttler and Schroff 1976) biochemical changes (Gnaiger 1980*a*). This problem was investigated further in a simultaneous calorimetric and biochemical study of *Mytilus edulis* (Shick et al. 1983), and it was shown that biochemical estimates of ATP turnover are significantly less than calorimetric rates (Gnaiger 1983*a;* Gnaiger et al., in preparation). The same conclusion of unexplained anoxic heat must be drawn from the data of Famme and Knudsen (1984) on *T. tubifex.* The measured heat change divided by the molar amount of excreted volatile fatty acids, VFA, was in the range −146 kJ/mol VFA. However, the theoretical enthalpy change is −73 and −132 kJ/mol acetic and propionic acid, respectively, since the acids were excreted and neutralization with environmental buffers is accompanied by a low enthalpy of neutralization compared with cellular buffers (table 1 in Gnaiger 1983*a*). Since 1.5 times more acetate than propionate was measured, the theoretical caloric equivalent is −97 instead of −146 kJ/mol VFA. Thus, the biochemically explained heat is only 66% of the calorimetric heat change. Caution is required in the quantitative interpretation of these data since the high acetate:propionate ratio of 1.5 (Famme and Knudsen 1984) contradicts any known biochemical mechanism in anoxic animals. For the maintenance of glycolytic redox balance, the maximum ratio of acetate:(succinate + propionate) is 0.5. This "acetate ratio" (Gnaiger 1980*b*) has a minimum theoretical value because for every mol acetate, 2 mol NADH are produced, and 2 mol fumarate must be reduced to succinate to reoxidize 2 mol NADH in turn (Gnaiger 1977). Reported anoxic acetate ratios of aquatic oligochaetes are ≦0.5 (Schöttler and Schroff 1976; Seu et al. 1983; Putzer 1985).

The biochemical estimates of anoxic en ergy expenditure in euryoxic invertebrate may be incomplete at present, but even les information is available on anoxic energ consumption and absorption. It might b expected that food intake under anoxia enhanced for compensation of the highe loss of combustion energy in anoxic AT production (see above). On the contrar cessation of feeding under anoxia (McCa and Fisher 1980) and even an induction b anoxia for emptying the gut is reported fo tubificids and *Lumbriculus* (Alsterber 1922). Observations of this behavior are no unique in oligochaetes. The biochemica adaptations of the isopod *Cirolana boreali* were studied, since "in nature this scaveng ing animal encounters low oxygen tensio when it burrows into the flesh of dead fis to feed"; however, it "responds to exper mental anoxia by emptying the stomac through the mouth" (De Zwaan and Skjo dal 1979). In contrast, filtration rates of th clam *Mulinia lateralis* at $P_{O_2} < 0.5$ kPa o <2.5% air saturation are the same as aerobi rates (Shumway, Scott, and Shick 1983). I is not known whether this high anaerobi filtration rate is partly related to the effor of extracting residual oxygen from the wate rather than to food consumption and an oxic assimilation. Calorimetric and respi rometric measurements of the mussel *My tilus edulis* exposed to air suggest tha incomplete valve closure and aerial oxyge uptake are correlated with some aspect o digestion or assimilation (Widdows an Shick 1985; Shick et al. 1986).

Unexpectedly, *L. variegatus* discontin ued defecation during anoxic starvation af ter only half of the gut was emptied (figs 5, 6). After a period of aerobic defecatio and anoxic acclimation, this oligochaete di feed under anoxia at a low rate and change the pattern of egestion (cf. figs. 7, 8). Growth of *Tubifex* sp. was observed in an anaerobi laboratory culture (Famme and Knudse 1985*b*). Aquatic oligochaetes are extremel effective in removing oxygen from the wate at P_{O_2} levels of 0.1 kPa or 0.5% air satu ration (fig. 4; Gnaiger, unpublished) Therefore, undetectable oxygen concentra tions in the *outflow* of the culture (Famm and Knudsen 1985*b*) are no proof that th

tubificids did not consume oxygen to supplement the ATP demand for growth. However, if strictly anoxic growth occurred, this would necessarily be dependent on a high rate of anoxic energy uptake. *Tubifex tubifex* in natural anoxic sediments does not grow and suffers high mortality (Graef 1985).

These apparent discrepancies may be resolved on the basis of concepts of scope for growth and optimum foraging theory. The metabolic cost of feeding increases with decreasing caloric values of the food. If the cost also increases with increasing feeding rate, then the optimum feeding rate, providing the maximum scope for growth, must decrease with a decreasing density of digestible organic matter (Bayne 1987). Moreover, low biochemical efficiency of maintenance metabolism owing to genetically determined high protein turnover decreases the feeding rate (Bayne 1987; Hawkins, Bayne, and Day 1986). Even more pronounced effects on optimum feeding rate must be expected under environmental anoxia when the stoichiometric (not ergodynamic) "efficiency" of ATP turnover is decreased 5.8-fold (see above). At low food densities, the metabolic cost of anoxic assimilation surmounts the energy gain, in which case active feeding entails a net energy loss relative to passive starvation. Anoxic feeding becomes a strategy superior to passive starvation only above a critical food concentration. Above an even higher threshold concentration, a positive anoxic energy balance is possible. This might explain why *Tubifex* sp. may grow under anoxia on a high-quality food (trout food pellets inoculated for >10 days; Famme and Knudsen [1985*b*]), and why we observed some anoxic feeding of *L. variegatus* using spinach homogenate enriched by 4–10 days of microbial growth, whereas *T. tubifex* in natural anoxic lake sediments with limited available energy does not show any measurable feeding activity (McCall and Fisher 1980). This concept should stimulate investigations into quantifying the anoxic threshold concentration of food in comparison with the aerobic threshold concentration sufficient for growth (Schiemer 1983). Such information is required to evaluate the physiological effect on benthic animals of anoxia and of toxic substances in anoxic sediments.

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