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Heat Dissipation and Energetic Efficiency in Animal Anoxibiosis: Economy Contra Power

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ABSTRACT This survey on calorimetry and thermodynamics of anoxibiosis applies classical and irreversible thermodynamics to interpret experimental, direct calorimetric results in order to elucidate the sequential activation of various biochemical pathways. First, the concept of direct and indirect calorimetry is expanded to incorporate the thermochemistry of aerobic and anoxic metabolism in living cells and organisms. Calorimetric studies done under normoxia as well as under physiological and environmental anoxia are presented and assessed in terms of ATP turnover rate. Present evidence suggests that unknown sources of energy in freshwater and marine invertebrates under long-term anoxia may be important.

During physiological hypoxia, thermodynamically grossly inefficient pathways sustain high metabolic rates for brief periods. On the contrary, under long-term environmental anoxia, low steady-state heat dissipation is linked to the more efficient succinate, propionate, and acetate pathways. In the second part of this paper these relationships are discussed in the context of linear, irreversible thermodynamics. The calorimetric and biochemical trends during aerobic-anoxic transitions are consistent with thermodynamic optimum functions of catabolic pathways. The theory predicts a decrease of rate with an increase of thermodynamic efficiency; therefore maximum rate and maximum efficiency are mutually exclusive. Cellular changes of pH and adenylate phosphorylation potential are recognized as regulatory mechanisms in the energetic switching to propionate production. While enzyme kinetics provides one key for understanding metabolic regulation, our insight remains incomplete without a complementary thermodynamic analysis of kinetic control in energetically coupled pathways.

Rapid muscular contraction is an explosive process, powerful but exhaustive. During periods of a few seconds, essentially anoxic biochemical pathways are employed (Kushmerick, '77; diPrampero, '81). On the other hand, the animals that survive long-term anoxia (von Brand, '46) seem to be living in "slow motion," using modes of energy release that have recently attracted much scientific interest (e.g., Hochachka, '80). The biochemical responses to environmental oxygen deprivation have been studied most carefully in the bivalve mollusk, *Mytilus edulis* (de Zwaan, '77) and in parasitic helminths (Saz, '81). Two disparate features characterize animal anoxibiosis: high power output in the case of physiological anoxia, and high efficiency of energy conversion under environmental anoxia. The contrast is reminiscent of the evolutionary distinction between "*r*-selected" (maximum growth rate) and "*K*-selected" (maximum sustaining capacity) species (MacArthur and Wilson, '67; Parry, '81). Are the types of anoxic metabolism essentially and necessarily different? The purpose of this paper is to analyze the distinction between high power and high energetic economy in animal anoxibiosis, in terms of basic thermodynamic principles.

Energy that is released in metabolism and is not used for work or biosynthesis is con-

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verted into heat. In this paper, the heat leaving an organism is called *dissipative*. It is readily measurable by direct calorimetry. However, even in frictionless ideal energy conversion, heat changes due to reversible structural rearrangements are possible. These reversible or *compensatory* heat changes are negligible in aerobic metabolism, but are quantitatively important in anoxic biochemical reactions. Anoxic heat dissipation, therefore, is not exactly equivalent to the irreversible degradation of free energy. This is recognized in muscle physiology (Burk, '29; Wilkie, '60), and is important in ecophysiological microcalorimetry of anoxic metabolism (Gnaiger, '80a).

In addition to a careful thermodynamic interpretation of heat, we wish to establish the comparability of aerobic and anoxic heat dissipation on a thermochemical and biochemical basis. For various pathways of anoxic catabolism, heat dissipation decreases more than ATP turnover, when compared with the aerobic processes (Gnaiger, '80a,b). Consequently, aerobic and anoxic rates of heat dissipation are not strictly comparable in terms of ATP turnover. Moreover, discrepancies have been observed between calorimetric (total) heat dissipation, $_t\dot{Q}$ and metabolic heat dissipation, $_m\dot{Q}$, as calculated from measured biochemical changes under anoxia:

$$\left| {}_t\dot{Q} \right| > \left| {}_m\dot{Q} \right| \qquad (1)$$

The involvement of some reactions related to ATP production has been suggested (Karnaukhov, '79; Zs.-Nagy, '77), but their quantitative role is not known at present.

The unique advantage of the calorimetric method is that it detects the enthalpy changes of all reactions, even if the nature of these reactions is not fully understood. However, the physiological interpretation of anoxic heat dissipation and the functional comparison of aerobic and anoxic heat changes cannot be completed until more biochemical information is obtained. The basic idea of using calorimetry to explore the *sources* and *dynamics* of heat changes was present in the origins of bioenergetics 200 years ago: "Respiration is therefore a combustion, a very slow one to be precise" (Lavoisier and LaPlace, 1783).

DIRECT AND INDIRECT CALORIMETRIC METHODS

Microcalorimetry

The instrumental aspects of microcalorimetric methods as applied to aquatic animals have been outlined in detail elsewhere (Gnaiger, '79, '83a; Pamatmat, '83).

Usually the design of the animal chamber sets an upper limit on size of experimental animals, and the sensitivity and long-term stability of the instrument set the lower limit. In studies of aerobic and anoxic metabolism I have used thermopile heat-conduction calorimeters with animal chamber volumes of 0.5 cm^3 (LKB 2107 flow sorption microcalorimeter), and 5 and 25 cm^3 (LKB BioActivityMonitor, 4-channel modular system) (Suurkuusk and Wadsö, '82). The experimental biomass ranged from 0.7 to 5 mg dry weight ($_dW$) in the former case (6–20°C) and from 10 to 600 mg $_dW$ (excluding the shell of mussels) in the latter (12–15°C).

The problem of instrumental time resolution and the necessity of mathematical corrections of the apparent rates is well recognized in calorimetry (Calvet and Prat, '63; Randzio and Suurkuusk, '80). The significance of exponential time constants has also received attention in open-flow respirometry (Gnaiger, '83a). However, the problem of time resolution in closed respirometric and adiabatic calorimetric systems has usually been ignored. Apparent and actual rates may differ significantly, owing to low flow rates in open systems, and because of the necessity of linear regression or interpolation in closed systems. Uncorrected results must be interpreted with care, if no information on the magnitude of experimental errors is available.

In simultaneous calorimetry and respirometry (Fig. 1) the response times are usually different for the two detector systems. The response time of the twin-flow microrespirometer was calibrated relative to the LKB flow-sorption microcalorimeter. The time constant, τ, was estimated to vary between 5 and 15 min at a flow rate of 0.095 $cm^3\ min^{-1}$, and was used to correct the instrumental output (Gnaiger, '83a).

Stoichiometry and thermochemistry of catabolism (indirect calorimetry)

Metabolic reaction rates are quantified on the basis of concentration changes of sub-

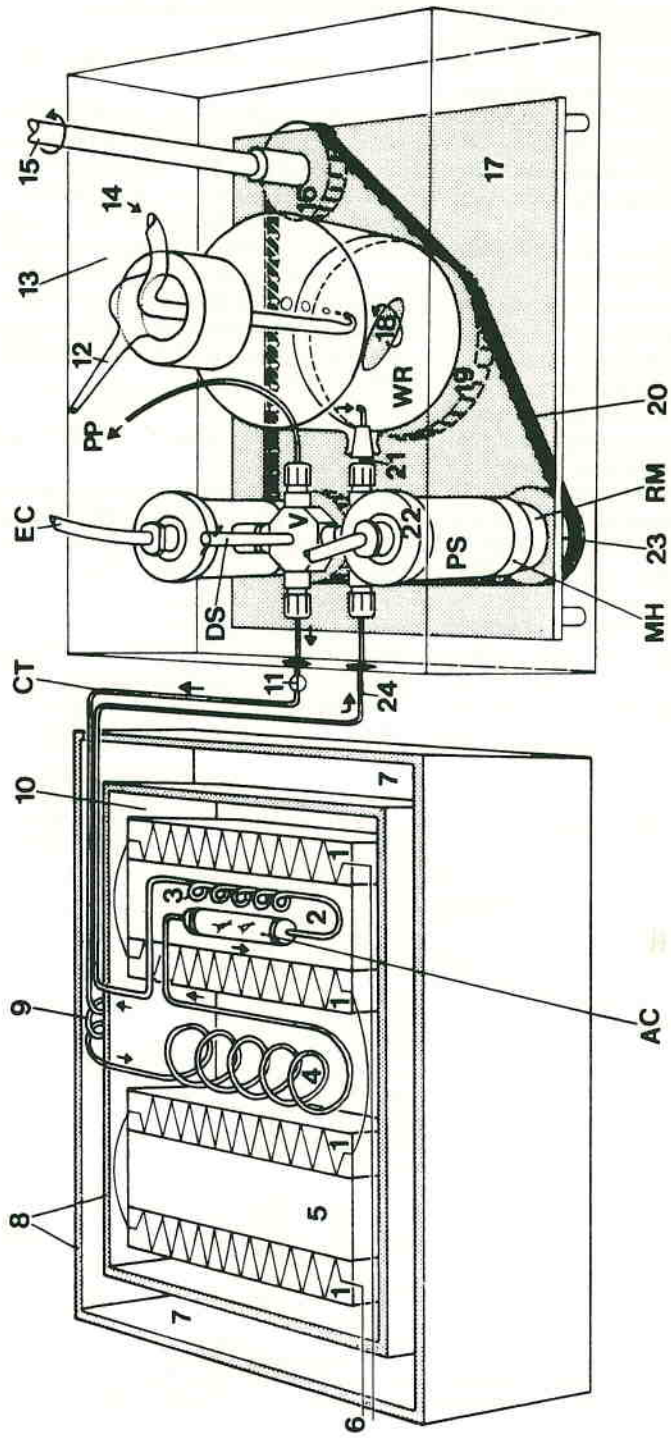

Fig. 1. The open-flow respirometer-calorimeter: the twin-flow microrespirometer (Cyclobios-Austria) connected to the LKB-2107 flow sorption microcalorimeter. AC, animal chamber; CT, capillary tube; DS, drive shaft for switching the two four-way valves; EC, electrode cables of the polarographic oxygen sensor; MH, magnet housing; PP, outlet Teflon tubing connected to the peristaltic pump; PS, polarographic oxygen sensor sleeve; RM, rotating magnet; V, four-way microvalve; WR, water reservoir for P_{O_2} equilibration (from Gnaiger, '83a).

strates, intermediates, and products. The same substance—e.g., succinate—may be an intermediate and a product at the same time. In the metabolic steady state, intermediates remain constant. Then the change of end products reflects the total catabolic rate, provided the respective pathways are known. The rate of catabolic ATP generation is calculated from experimentally established coupling coefficients which are specific for each pathway (Table 1).

Under normal physiological conditions the rate of ATP generation is balanced by ATP consumption. In aerobic-anoxic transitions, however, total ATP consumption is complemented by changes of adenylate concentrations, depletion of phosphagen levels, and presumably also by changes in chemiosmotic potentials. During aerobic recovery, these reactions are reversed, and original concentration levels are restored. Because of their importance under physiological and ecological hypoxia, it is convenient to summarize these reactions (net changes in ATP, AMP, phosphoarginine or phosphocreatine, etc.) by the term "*ergo*bolic" reactions. Heterotrophic energy metabolism is thus subdivided into catabolism (k), ergobolism (e), and anabolism (a). Metabolic heat dissipation, ${}_{m}\dot{Q}$ [mW], is calculated, under isothermal conditions, as the sum of the respective enthalpy changes per unit time:

$$ {}_{m}\dot{Q} = {}_{k}\dot{Q} + {}_{e}\dot{Q} + {}_{a}\dot{Q} - \dot{W} \qquad (2) $$

$\dot{W}$ is the mechanical power which is normally zero in calorimetry, since no work appears outside the apparatus. Moreover, anabolism is likely to be inhibited during severe hypoxia in free-living animals, in which case metabolic heat dissipation consists only of two terms, ${}_{k}\dot{Q}$ and ${}_{e}\dot{Q}$.

Catabolic heat dissipation, ${}_{k}\dot{Q}$ [mW], is calculated from aerobic and anoxic rates of catabolism combined with the respective molar enthalpy changes:

$$ {}_{k}\dot{Q} = \dot{N}_{O_2} \times \Delta_k H_{O_2} + \dot{N}_p \times \Delta_k H_p \qquad (3) $$

The rates of catabolic reactions are expressed as $\dot{N}_{O_2}$ and $\dot{N}_p$ (μmol s^{-1}), the rate of oxygen consumption and anoxic endproduct formation, respectively. $\Delta_k H_{O_2}$ [kJ (mol O_2)$^{-1}$, or mJ (μmol O_2)$^{-1}$] is the oxycaloric equivalent, more explicitly defined as the caloric equivalent of oxygen consumption in the catabolic half cycle or in dissipative metabolism (Gnaiger, '83b). Although $\dot{N}_{O_2}$ is a negative

TABLE 1. Oxycaloric equivalents, $\Delta_k H_{O_2}$, caloric equivalents of anoxic end products, $\Delta_k H_p$, and enthalpy changes in ergobolic reactions, $\Delta_e H_{ATP}$, in environmental buffers (for excreted acids, pH 6 to 8) and body fluids (for accumulated acids, pH 7)[1]

	Environmental		Body fluids		
$\Delta_b H'$ (H+	0	−8	−15	−25	
Aerobic[2]: $\Delta_k H_{O_2}$ (kJ mol^{-1})					ν_{ATP/O_2}
Glycogen	−471	−478			6.17
Lipid	−440	−445			5.69
Protein (→NH_4^+)	−447	−451			5.25
Anoxic[3]: $\Delta_k H_p$ (kJ mol^{-1})					$\nu_{ATP/p}$
Lactate (glucose)	−55	−63	−70	−80	1.00
Lactate (glycogen)	−56	−64	−71	−81	1.50
Ethanol	−63	−71	−76	−85	1.50
Alanine (+ Succ)			−32	−40	0.00
Alanine			−93	−103	1.50
Succinate			−136	−152	2.75
Acetate (+ Succ, Prop)	−65	−73	−80	−90	2.00
Propionate	−120	−132	−142	−156	3.75
Ergobolic: $\Delta_e H_{.p}$ (kJ mol^{-1})					
ATP→ADP[3]			−38	−46	
P-creatine hydrolysis[4]				−34	

[1]The anoxic substrate is glycogen, but also glucose in the case of lactate. Alanine can also be formed by transamination from aspartate [alanine (+ Succ)] coupled to succinate production at a ratio of 1.17:1. ν_{ATP/O_2} and $\nu_{ATP/p}$ are the stoichiometric ATP coupling coefficients per mole of oxygen and product, respectively, for fully coupled systems.
[2]From Gnaiger ('83b).
[3]From Gnaiger ('80a and unpublished).
[4]From Woledge ('72).

quantity, the convention of regarding oxygen uptake as positive is followed in this paper.

The ratio of total heat dissipation and oxygen consumption is the (total) heat equivalent of oxygen, $\Delta_t Q_{O_2}$ [kJ (mol O_2)$^{-1}$]; it is the experimental counterpart to the theoretically derived $\Delta_k H_{O_2}$ value (compare Eq. 3):

$$\Delta_t Q_{O_2} = \frac{\dot{Q}}{\dot{N}_{O_2}} \qquad (4)$$

If $\Delta_t Q_{O_2}$ exceeds the range of theoretical $\Delta_k H_{O_2}$ values (Table 1), then partial anoxibiosis is indicated (Eq. 3). It is noteworthy that the classical indirect calorimetric approach (Brody, '45; Kleiber, '61) is based on the oxycaloric equivalent, $\Delta_k H_{O_2}$, and considers aerobic catabolic half cycle reactions only: net ergobolic or anabolic reactions are not accounted for.

Likewise, $\Delta_k H_p$ [kJ (mol product)$^{-1}$] is the caloric equivalent of the average anoxic end-product in dissipative metabolism. $\Delta_k H_{O_2}$ ranges from -445 to -478 kJ mol^{-1}, while $\Delta_k H_p$ ranges from -65 to -156 kJ mol^{-1} (Table 1). Under hypoxia, low rates of oxygen consumption can contribute significantly to heat dissipation, since the molar enthalpy change for oxygen is three to seven times higher than for anoxic end products.

The calculation of molar enthalpy changes of biochemical reactions under physiological conditions involves some complexities which have caused unnecessary confusion in the literature on animal anoxibiosis. Standard reaction enthalpies in dilute aqueous solution at pH 7, $\Delta_r H^{o\prime}$, are obtained from the respective enthalpies of formation, $\Delta_f H^{o\prime}$ (Wilhoit, '69). However, biological fluids are buffered solutions. Hence a fraction, x_{H+}, of the hydrogen ions that are evolved concomitantly with the accumulation of acidic end products undergoes a side reaction with the buffers. This side reaction is associated with an additional enthalpy change which may contribute up to 40% of the overall heat effect of anoxic catabolism and ergobolic reactions (Woledge, '72; Gnaiger, '80b).

The physiological enthalpy change of a catabolic anoxic reaction is therefore calculated as

$$\Delta_k H_p = \Delta_k H'_p + \nu'_{H+} \times \Delta_b H'_{H+} \qquad (5)$$

where ν'_{H+} is the stoichiometric coefficient of proton evolution at the prevailing pH, and $\Delta_b H'_{H+}$ is the apparent enthalpy change of the buffer system [kJ (mol H^+ added)$^{-1}$]. In the latter term x_{H+} and the enthalpy change of the buffer, $\Delta_b H_{H+}$ [kJ (mol H^+ buffered)$^{-1}$], are combined:

$$\Delta_b H'_{H+} = x_{H+} \times \Delta_b H_{H+} \qquad (6)$$

Quite large uncertainty ranges have to be assigned to $\Delta_b H'_{H+}$, if specific information about the buffering processes is lacking. $\Delta_b H_{H+}$ varies from positive values near zero to values below -30 kJ mol^{-1} for the buffer systems that are most important in organisms (Woledge, '72; Gnaiger, '80b). Since the protonation enthalpies of cellular buffer systems are generally more negative than those of buffer systems in the aquatic environment, the catabolic heat effects decrease (become less negative) with an increasing fraction of excreted versus accumulated organic acids.

Realistic ranges of $\Delta_b H'_{H+}$ may be estimated at -15 to -25 kJ mol^{-1} for accumulated acids and at 0 to -8 kJ mol^{-1} for excreted acids. A special situation arises in the case of animals undergoing anoxia by closing their calcareous shells. Carbonate dissociation is then involved in buffering the internally excreted acids (Crenshaw and Neff, '69; Gordon and Carriker, '78), but the bicarbonate/carbon dioxide system tends toward equilibrium and neutralizes a fraction of the organic acids at the same time.

$$H^+ + CaCO_3 \rightarrow Ca^{++} + HCO_3^-; \qquad (7a)$$
$$\Delta_b H_{H+} = -28 \text{ kJ mol}^{-1}$$

$$H^+ + HCO_3^- \rightarrow H_2O + CO_2(aq); \qquad (7b)$$
$$\Delta_b H_{H+} = -8 \text{ kJ mol}^{-1}$$

The $\Delta_b H'_{H+}$ values are given for 25°C (Wilhoit, '69) and are more negative at lower temperatures. However, submaximal apparent buffer enthalpies, $|\Delta_b H'_{H+}| < |-25$ kJ mol$^{-1}|$ are to be expected in this situation.

EXPERIMENTAL RATES OF AEROBIC AND ANOXIC HEAT DISSIPATION

Sections of a calorimetric experiment with the aquatic oligochaete *Lumbriculus variegatus* are shown in Figure 2. The water was drawn through the perfusion chamber at a flow rate of 3.3 cm^3 h^{-1} and was previously stripped of oxygen by pure nitrogen or a mixture of 8% CO_2 in N_2, to obtain heat dissipation under *environmental* anoxia. The anoxic power-time curve was stable at about 25% of

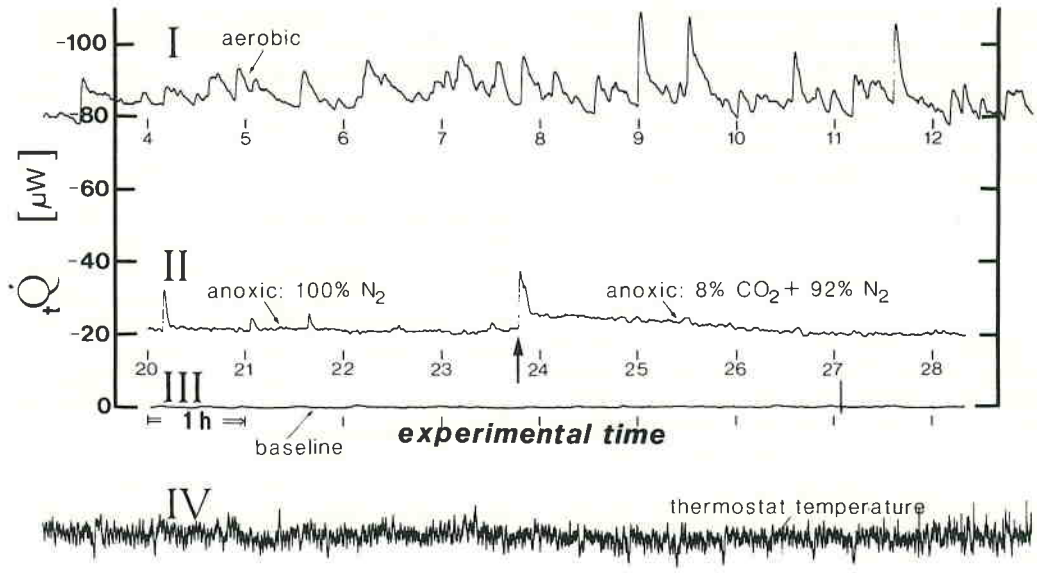

Fig. 2. Superimposed segments of a calorimetric experiment (LKB-2107 flow sorption microcalorimeter) showing aerobic and anoxic rates of heat dissipation of a group of ten *Lumbriculus variegatus* (94.1 mg total $_w W$; 1.60 mg $_d W$ per individual). The traces show (from top to bottom) I: Aerobic rate with irregular activity pattern; the time of the experiment, 4–12 h, is indicated by numbers. II: Anoxic steady-state rate recorded for 46 h (20–28 h of anoxia shown, corresponding to 60–68 h of the experiment); the water was equilibrated with 100% N_2 or 8% CO_2 in N_2; the switching to the gas mixture (arrow) induced a peak of heat dissipation which might be due to the temporal intrusion of some oxygen; the stable rates indicated no effect of CO_2. III: After poisoning the animals (Amoquar, 0.1%) a stable baseline was reached within 20 h; long-term stability was observed in the following 95 h (8 h shown) at ± 1 μW. IV: Continuous record of thermostat temperature (see Kaufmann and Gnaiger, '81) at 20°C. The constant perfusion rate through the 0.5 cm^3 animal chamber was 3.3 $cm^3\ h^{-1}$.

aerobic heat dissipation without a conspicuous activity pattern. Fluctuations in heat output are common in aerobic animals in heat-conduction calorimeters and open-flow respirometers, where the steady-state signal is directly proportional to the rate.

Between periods of high activity, a relatively constant level of heat dissipation is frequently observed and has been interpreted as basal metabolism (Pamatmat, '80). This is not in accord with the physiological definition of basal metabolism; there may be a constant activity component involved in the apparent minimum rate, which may be termed "level routine rate" to distinguish it from the total (integrated and averaged) routine rate. The difference between the two will be referred to as "(average) increment of routine activity." The activity pattern has been discussed in relation to parasitic infections (Becker and Lamprecht, '77; Becker, '80) and toxicological effects (Gnaiger, '81). Discontinuous bursts of metabolic activity may be sustained in part by anoxic mechanisms (*physiological* hypoxia).

Physiological hypoxia

During physiological hypoxia the rate of heat dissipation is typically in a nonsteady state. Distortion of the pattern can be avoided by mathematical time correction or by integrating the power-time curve over extended periods. In *L. variegatus* the average increment of routine activity amounted to only 10% of total routine rates, independent of oxygen and temperature (Fig. 3). Therefore, physiological anoxia was not important during these conditions. This conclusion is also supported by the agreement of calorimetric and respirometric rates of the oligochaetes under air saturation (Gnaiger, '80c, '83a). In a bivalve species, however, the increment of routine activity averaged 30% of total heat changes (Pamatmat, '80, '83).

Physiological hypoxia cannot be discerned from purely aerobic mechanisms by direct

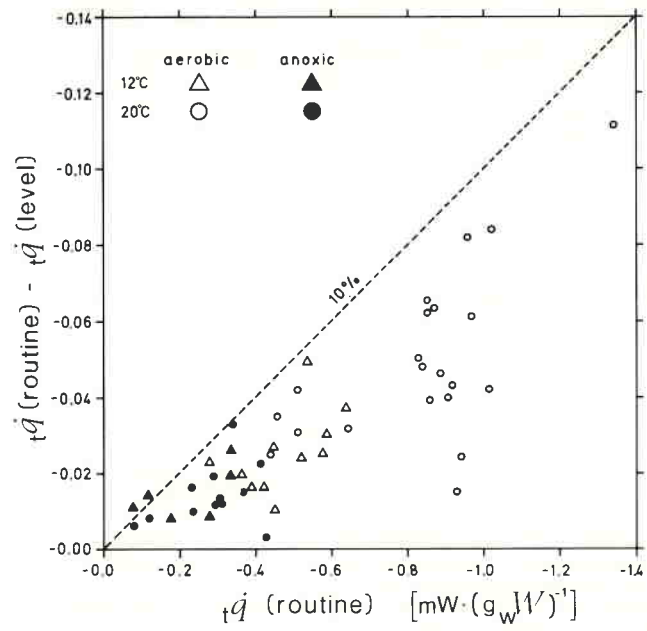

Fig. 3. The "average increment of routine activity," $_t\dot{q}$ (routine) − $_t\dot{q}$ (level), in relation to the specific routine rate of heat dissipation, $_t\dot{q}$ (routine), in *Lumbriculus variegatus* under aerobic and anoxic conditions, and at two temperatures.

calorimetry alone; the simultaneous measurement of oxygen consumption is required (Fig. 1). The correlation of high experimental heat equivalents of oxygen, $\Delta_t Q_{O2}$, with high total heat dissipation rates may indicate a share of anoxic metabolism during periods of spontaneous burst activity (Fig. 4). In larvae of the arctic charr *Salvelinus alpinus*, this was certainly the case during stress-induced activity (Fig. 5; addition of streptomycin and neomycinsulfate, 0.2 g dm^{-3}), when $\Delta_t Q_{O2}$ increased from −440 to −600 kJ (mol O_2)$^{-1}$. During this period of physiological hypoxia about 25% of total heat dissipation was contributed from anoxic sources (Gnaiger, '81).

Physiological hypoxia is not restricted to locomotory burst activity, but may complement aerobic respiration during periods of elevated metabolic energy demand in general. This has been suggested on the basis of biochemical estimates of anoxic rates during recovery from anoxic exposure (aerobic overshoot) in the adductor muscle of *Mytilus edulis* (deZwaan et al., '83). During recovery, level routine rates of *L. variegatus* are increased up to 40% of the aerobic steady-state heat dissipation (Fig. 6), which is partly due to the formation of anoxic end products (V. Putzer, personal communication).

Environmental anoxia

Whereas physiological hypoxia is combined with maximum rates of heat dissipation, rates decrease to minimum values under environmentally induced anoxia (Fig. 5). In animals that tolerate the lack of oxygen for an extended period of time, heat dissipation approaches anoxic steady-state levels (Figs. 2, 7) which may vary between 7% and 60% of aerobic rates (Famme et al., '81; Gnaiger, '79, '80a,c; Hammen, '80; Pamatmat, '79, '80, '83; Shick, '81). Weight-specific rates of steady-state anoxic heat dissipation range from −0.05 to −1.8 mW ($g_d W$)$^{-1}$.

Intraspecific variability of the anoxic/aerobic ratio is a function of temperature and increases with the level of steady-state activity in *L. variegatus* (Gnaiger, '80c). The ecophysiological interpretation of interspecific differences must be largely speculative until a wider range of studies becomes available for comparison of aerobic and anoxic rates under standardized physiological conditions. Intertidal species undergoing partial anoxia

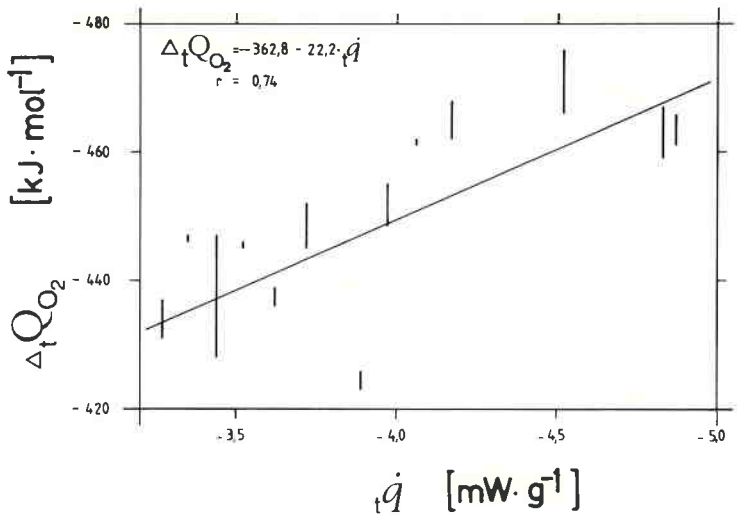

Fig. 4. Simultaneous calorimetry and respirometry of *Salvelinus alpinus* during spontaneous fluctuations of activity at normoxia. The experimental caloric equivalent of oxygen consumption, $\Delta_t Q_{O2}$ (heat dissipated per mole of oxygen consumed), as a function of the specific rate of heat dissipation, ${}_t\dot{q}$, averaged over 20-min periods. The bars indicate the ranges of instantaneous values of $\Delta_t Q_{O2}$ due to activity-dependent variations of the respirometer time constant.

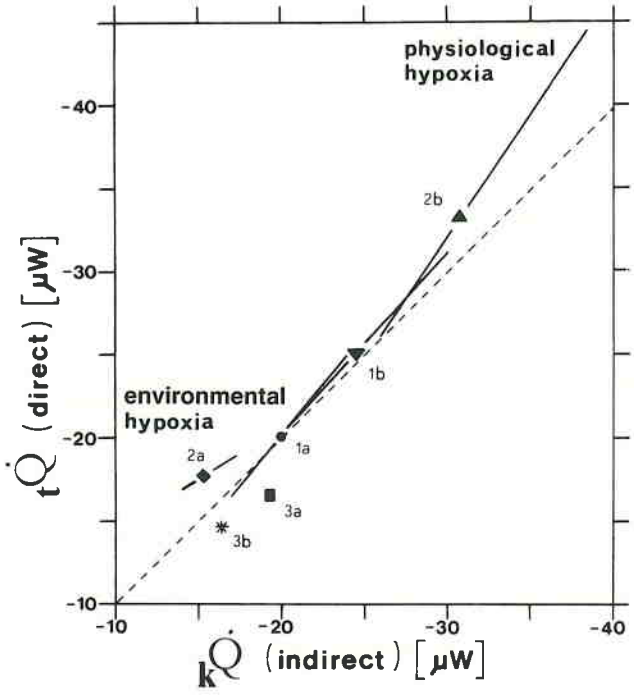

Fig. 5. Relation of direct, ${}_t\dot{Q}$, and indirect, ${}_k\dot{Q}$, calorimetry in *Salvelinus alpinus* (8°C; after hatching, 5.11 mg ${}_dW$). The broken line shows the theoretical relation for purely aerobic dissipative catabolism, $\Delta_k H_{O_2} = -450$ kJ (mol O_2)$^{-1}$. The symbols indicate the mean rates during specified periods and the solid lines are the corresponding regressions (Bartlett's method of best fit) corrected for instrumental lag. 1) Balanced aerobic dissipative metabolism during (1a) normoxia (see Fig. 4) and (1b) after acclimation to hypoxia and antibiotics (48% air saturation). 2) Mixed aerobic and anoxic metabolism during (2a) acute exposure to *environmental hypoxia* (48% air saturation, anoxic compensation period) and (2b) stress induced *physiological hypoxia* (acute exposure to antibiotics). 3) Apparent conservation of dissipative heat loss (possibly due to ergobolic and anabolic reactions; see Eq. 2) following anoxic compensation periods (3a) at 48% and (3b) at 19% air saturation (from Gnaiger, '83a).

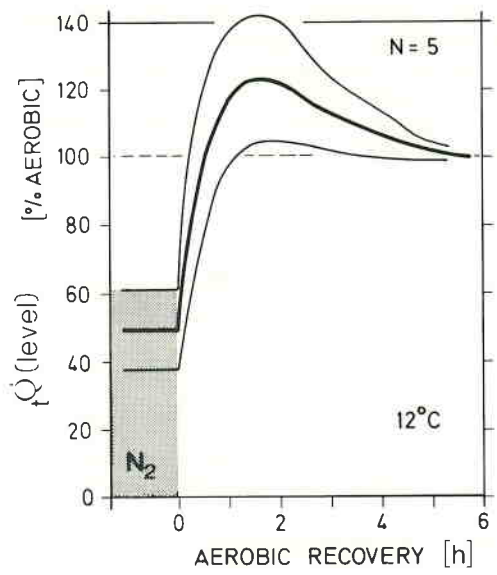

Fig. 6. Heat dissipation of *Lumbriculus variegatus* during environmental anoxia (stippled area) and during the anoxic-aerobic transition (aerobic overshoot). The thick line represents the mean level rate in five experiments, and the thin lines are the boundaries of ± 1 S.D. Part of the high rate during the overshoot is due to anoxic metabolism (from Gnaiger, '80b).

during air exposure (Fig. 7) exhibit low anoxic/aerobic heat dissipation ratios (Famme et al., '81; Pamatmat, '80, '83; Shick, '81). Some intertidal species depend more on aerial oxygen availability than on anoxic metabolism. Others save energy by becoming inactive in air. However, any evaluation of anoxic "energy saving" (Pamatmat, '80) must take into account the cost of postanoxic recovery (Shick, '81; Widdows et al., '79). We do not know whether some species require a higher maintenance rate than others for mere anoxic survival, or whether the relatively higher rates (e.g., of the oligochaetes) are related to some other functional aspects of anoxic metabolism (digestion, biosynthesis). The metabolic flexibility allowing for a reduction of energy expenditure is a prerequisite for long-term tolerance to anoxia, especially at high temperature (Gnaiger, '80c).

THERMODYNAMIC AND BIOCHEMICAL INTERPRETATION OF AEROBIC AND ANOXIC HEAT DISSIPATION

Aerobic and anoxic energy balance

In experiments employing heat-flow calorimetry and simultaneous respirometry, the

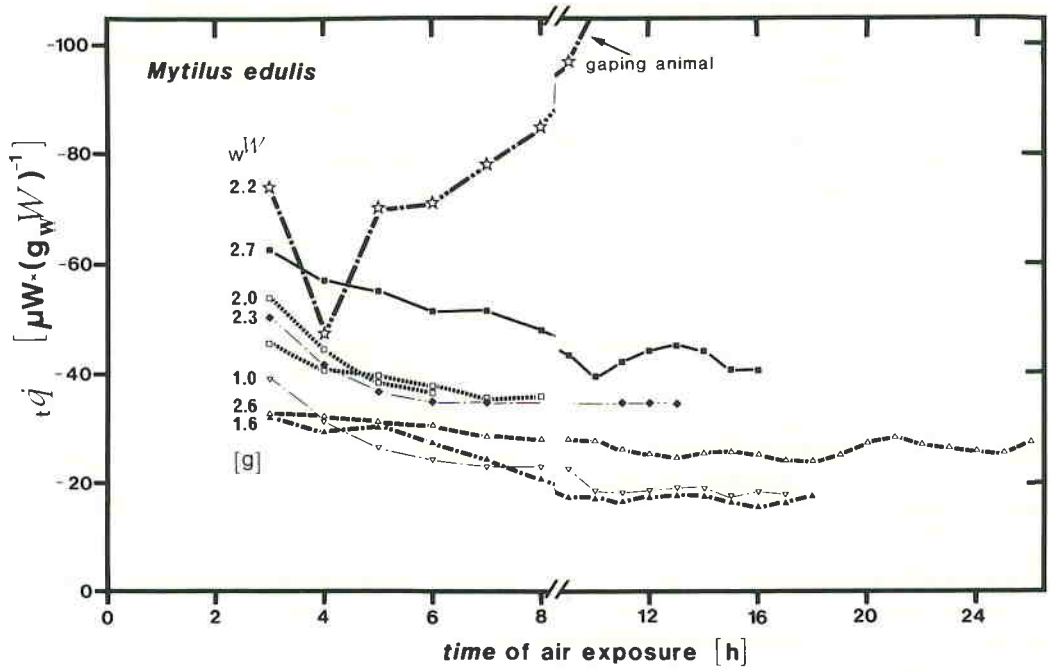

Fig. 7. Aerobic-anoxic transition in *Mytilus edulis* during air exposure (15°C; 1.0–2.7 g $_w W$), measured in a 25 cm^3 stainless steel chamber of a prototype LKB BioActivityMonitor microcalorimeter. The symbols indicate mean routine rates averaged over 1-h intervals.

experimental (total) heat equivalents of oxygen consumption, $\Delta_t Q_{O_2}$ (see Eq. 4), approximate -450 kJ (mol O_2)$^{-1}$ for freshwater and marine animals including gastropods, bivalves, crustacea, and fish (Famme et al., '81; Gnaiger, '83a; Lowe, '78; Pamatmat, '78, '83). The simultaneous results are in accord with the theoretical oxycaloric equivalent (Table 1). In nonsimultaneous measurements of heat dissipation and oxygen uptake, different states of activity of the animals in the calorimeter and respirometer may explain the large range of experimental $\Delta_t Q_{O_2}$ values (Becker and Lamprecht, '77; Coenen-Stass et al., '80; Dunkel et al., '79; Gnaiger, '80c; Peakin, '73; Shick '81) (for other explanations see Eq. 2). In adiabatic calorimeters (Dewar flasks), high rates of heat dissipation relative to oxygen consumption ($\Delta_t Q_{O_2} < -800$ kJ mol $^{-1}$) have been interpreted as indicating high anoxic rates under aerobic conditions (Grainger, '68; Hammen, '80), but these results may contain experimental artefacts (see Hammen, '83).

Rates of heat dissipation of the nematode parasite *Ascaris* under anoxia were measured by Krummacher ('19), and amounted to -0.8 mW $(g_d W)^{-1}$ at 38.5°C, in the same range as values for free-living invertebrates.

In long-term anoxia, following the aerobic-anoxic transition, ergobolic reaction rates become relatively unimportant (Ellington, '81; Meinardus and Gäde, '81; Wijsman, '76). Then, the approximation $_m\dot{Q} \approx {}_k\dot{Q}$ is biochemically justified (Eq. 2). The nonsimultaneous comparisons of direct calorimetric and indirect biochemical estimations of heat dissipation suggest that a large fraction of total enthalpy changes remains unexplained in anoxic metabolism (Gnaiger, '80a,c).

In a simultaneous calorimetric and biochemical experiment with *Mytilus edulis*, Shick et al. ('83) reported a large but statistically insignificant difference between the two methods, at least during the initial period of anoxibiosis. However, a recalculation of their data, employing more realistic enthalpy equivalents (Table 1), indicates that total anoxic heat dissipation of *M. edulis* is indeed significantly higher than can be accounted for by the measured biochemical reactions. Dissolution of shell $CaCO_3$ cannot explain this discrepancy.

Indirect calorimetry represents accurately the aerobic heat dissipation (for studies on mammals see Blaxter, '67; Brody, '45; Farrell, '74; Kleiber, '61; Rubner, 1894), perhaps with a trend to overestimate heat by the oxygen consumption measurement in some invertebrates (Lamprecht and Zotin, '78; Wieser and Gnaiger, '80). On the other hand, indirect calorimetry of euryoxic animals seems to underestimate total rates of anoxic heat dissipation (Gnaiger, '80a,c; Shick et al., '83). In anoxic vertebrate muscle (Curtin and Woledge, '78, '79; Homsher and Kean, '78) and in glycolytic human erythrocytes (Minakami and Verdier, '76; Spink and Wadsö, '76) observed heat changes are also higher than can be explained biochemically.

Heat dissipation and dissipative ATP turnover

Is there any physiological function associated with the anoxic extra heat? An attempt to interpret anoxic heat dissipation in functional terms must rely upon known catabolic and ergobolic mechanisms of ATP generation and consumption. Application of the following analysis to total heat changes ignores the possibility of an anoxic extra heat, and may therefore require further revision.

The rate of ATP production or utilization is taken here as equal to "metabolic rate." Oxygen consumption rate has been used synonymously with metabolic rate, but this usage is restricted to aerobic metabolism. The rate of heat dissipation is a general quantity that can be applied to aerobic and anoxic situations, but no useful function is known for most poikilotherms: Heat is a waste product of metabolism. How is heat dissipation related to the functional bioenergetics of ATP production and utilization?

Oxygen consumption and anoxic end-product formation are converted to catabolic ATP generation by employing stoichiometric ATP coupling coefficients (Table 1). Catabolic ATP generation in linear pathways involves simple coupling stoichiometries—e.g., three moles ATP per mole glycosyl unit in lactate fermentation, $\nu_{ATP/Glyc} = 3$ (Krebs and Kornberg, '57). The ATP coupling coefficients of branched pathways (succinate and propionate formation from glycogen), however, have not been firmly established (Gnaiger, '77; Hochachka et al., '73; de Zwaan, '77). An accurate calculation requires recognizing the specific pathways and formulating balanced reaction stoichiometries. The stoichiometric ATP coupling coefficients per mole of glycogen ($\nu_{ATP/Glyc}$) are 4.71 and 6.43, for succinate and propionate fermentation, respectively. No additional ATP is gained in the

formation of alanine, if aspartate-alanine transamination is coupled to the formation of succinate. In the 1:2 coupled acetate-succinate and acetate-propionate pathway $\nu_{ATP/Glyc}$ is 5.0 and 6.33, respectively. For both pathways of coupled acetate formation, the ATP yield per mole of acetate is 2. Substrate-based coupling coefficients are converted to product-based coefficients, $\nu_{ATP/p}$, by dividing $\nu_{ATP/Glyc}$ by the (negative) stoichiometric coefficient of the product in the balanced reaction equation (see Tables 1, 2).

The rate of catabolic ATP generation, $\dot{N}_{ATP}$, is calculated as

$$\dot{N}_{ATP} = \nu_{ATP/O_2} \times \dot{N}_{O_2} + \nu_{ATP/p} \times \dot{N}_p \quad (8)$$

where ν_{ATP/O_2} (= ~P/O_2 ratio) is the stoichiometric coupling coefficient of ATP for oxygen (compare Eq. 3). If neither (net) ergobolic nor anabolic reactions occur, then the rate of ATP generation and consumption are equal and are thus equivalent to the rate of dissipative ATP turnover, $\dot{N}_{\infty ATP}$. The catabolic heat dissipation, ${}_k\dot{Q}$[mW = mJ s^{-1}], divided by the rate of dissipative ATP turnover, $\dot{N}_{\infty ATP}$ [(μmol ATP) s^{-1}], yields the caloric equivalent of dissipative ATP turnover, $\Delta_k H_{\infty ATP}$ [mJ (μmol ∞ATP)$^{-1}$ = kJ (mol ∞ATP)$^{-1}$]:

$$\Delta_k H_{\infty ATP} = \frac{{}_k\dot{Q}}{\dot{N}_{\infty ATP}} \quad (9)$$

By dividing the aerobic and anoxic component of Eq. 3 by Eq. 8 we obtain the aerobic (ox) and anoxic (anox) caloric equivalents of dissipative ATP turnover more directly (Gnaiger, '80b):

$$\Delta_k H_{\infty ATP}(ox) = \frac{\Delta_k H_{O_2}}{\nu_{ATP/O_2}} \quad (10.1)$$

$$\Delta_k H_{\infty ATP}(anox) = \frac{\Delta_k H_p}{\nu_{ATP/p}} \quad (10.2)$$

$\Delta_k H_{\infty ATP}$ is the quantity providing the theoretical link between heat dissipation and ATP turnover in dissipative metabolism. Equivalence of the anoxic/aerobic ratio of heat dissipation and the anoxic/aerobic ratio of "metabolic rate" (of ATP turnover) depend on the similarity of $\Delta_k H_{\infty ATP}$ (anox) and $\Delta_k H_{\infty ATP}$(ox). For catabolic reactions known to predominate in euryoxic animals, however, the caloric ATP equivalent drops significantly during the aerobic-anoxic transition. Even at a constant "metabolic rate" (of ATP turnover) the rate of heat dissipation would decrease by up to 50% of the aerobic value. Therefore, a fraction of the generally observed reduction of anoxic relative to aerobic rates of heat dissipation can be predicted on the basis of this thermodynamic analysis (Fig. 8).

In burst activity, lactate (or equivalent end-product) formation is kinetically favored. When derived from free glucose, the catabolic enthalpy per mole of lactate ($\Delta_k H_p$) is −70 to −80 kJ mol^{-1}. One mole ATP is gained per mole lactate, $\nu_{ATP/p}$ = 1. In this case, according to Eq. 10.2, $\Delta_k H_{\infty ATP} = \Delta_k H_p$. This caloric equivalent of ATP turnover is not significantly different from aerobic values. However, if glycogen is the substrate, the heat dissipated per mole of ATP turnover is 30% less than for glucose. This is due to the thermodynamic property of glucosidic linkages, the hydrolysis of which proceeds with little enthalpy change, only −3.1 kJ mol^{-1} (Gnaiger, '80b), whereas the large free energy change drives the phosphorylation of 1 mole of ATP. The catabolic enthalpy of lactate formation is greater by −1.55 kJ mol^{-1} ($\Delta_k H_p$ = −71.5 to −81.5 kJ (mol lactate)$^{-1}$). The stoichiometric ATP coupling coefficient is 1.5 instead of 1, wherefore $\Delta_k H_{\infty ATP}$ amounts to −47.6 to −54.3 kJ (mol ATP)$^{-1}$ according to Eq. 10.2.

In the comparison of the physiological range of aerobic and anoxic $\Delta_k H_{\infty ATP}$ (Fig. 8), a fractional ATP coupling coefficient, x_{ATP}, of unity was used, the maximum of a coupled system. Lower degrees of coupling, $x_{ATP} < 1$, correspond to lower ~ P/O_2 ratios, $\nu_{ATP/O_2} \times x_{ATP}$ (Caplan, '71; Mitchell, '79; Rottenberg, '79; Zaba, '83). By definition, decoupling exerts no effect on the oxycaloric equivalent of dissipative metabolism. As Eq. 10.1 shows, therefore, decoupling would further increase the aerobic ratio of heat dissipation and ATP turnover, and hence magnify the difference between the aerobic and anoxic heat equivalent of ATP turnover (Fig. 8).

Correspondence between direct calorimetric and biochemical methods has been claimed for *Mytilus* on the basis of the observation that anoxic heat dissipation and ATP demand are reduced by a similar percentage of the respective aerobic rates (Pamatmat,

TABLE 2. Bioenergetic variables of aerobic and anoxic catabolism of glycogen

A[1]		cLactate	cAla	$c$$NH_4$	cSuc	cAcet	cProp	p_{CO_2}			$c$$P_i$	$\Delta_e G_{ATP}$
		[mmol dm^{-3}]						[atm]	pH	ATP/ADP	[mmol dm^{-3}]	[kJ mol^{-1}]
I		0.5	2	1	0.01	0.1	0.5	0.03	7.0	5	1	−51.1
II		5	8	1	5	2	2	0.05	6.5	1.5	3	−44.4
B[2]			uATP/S	ΔG_S / $\Delta_k G_{=ATP}$ [kJ mol^{-1}]			η^*	$\dot{N}_{ATP}$	$_e\dot{S}\cdot\eta^*$ [%]	$_e\dot{S}\cdot\eta^*\cdot\kappa$		σ
Aerobic	I		37.0	−2,879		−78	0.66	100	100			0.995
Anoxic												
Lactate	I		3.0	−254		−85	0.60	128	115	100		0.64
	II			−236		−79	0.56	131	96	83		0.69
Alanine	I		3.0	−236		−79	0.65	104	102	88		0.87
	II			−223		−74	0.60	112	89	77		0.92
Succinate	I		4.71	−331		−70	0.73	71	79	170		0.79
	II			−298		−63	0.70	71	65	140		0.88
Suc-acet	I		5.0	−345		−69	0.74	68	76	183		0.76
	II			−313		−63	0.71	69	64	154		0.84
Prop-acet	I		6.33	−372		−59	0.87	29	38	146		0.72
	II			−360		−56	0.79	45	46	182		0.69
Propionate	I		6.43	−365		−57	0.90	21	29	115		0.73
	II			−352		−55	0.81	39	41	165		0.69

[1]A: Cellular conditions during initial (I) and long-term (II) anoxibiosis (combined from Ebberink and deZwaan, '80; Ellington, '81; Gäde, '75; Gäde et al., '75; Gruner and Zebe, '78; Kluytmans et al., '78; Putzer, unpublished; Surholt '77a,b; Schöttler, '78, '79; Wijsman, '75, '76; Zurburg and Kluytmans, '80; de Zwaan et al., '83). c: Cellular concentrations of anoxic end products and inorganic phosphate, P_i; p_{CO_2}: partial pressure of CO_2 (1 atm = 101.325 kPa); $\Delta_e G_{ATP}$; Gibbs energy change of ATP hydrolysis calculated according to Alberty ('69) as a function of the ATP/ADP ratio, c_{P_i} and pH at pMg = 2.7

[2]B: Measures of efficiency, output power, and economy of aerobic catabolism and various anoxic pathways for initial (I) and long-term (II) anoxic conditions; alanine: formation from glycogen; suc-acet and prop-acet: succinate and acetate or propionate and acetate accumulation, respectively, in the ratio of 2:1 yielding a balanced NAD/NADH stoichiometry (Gnaiger, '77); $\nu_{ATP/S}$: "biochemical efficiency," stoichiometric coefficient of ATP yield per mole of glycosyl-unit catabolized; $\Delta_k G_S$ and $\Delta_k G_{=ATP}$: Gibbs energy changes per mole of glycosyl-unit and per mole of dissipative ATP turnover, respectively (Eq. 25 recalculated after Gnaiger ('77), with a Gibbs energy for the hydrolysis of a glucosidic linkage of −29.1 kJ mol^{-1}, with Gibbs energies of formation listed by Thauer et al. ('77), and for the conditions I and II specified in A); η^*: ergobolic/catabolic force ratio, thermodynamic efficiency of the fully coupled process (Eq. 18); $\dot{N}_{ATP}$: relative rate of catabolic ATP production (Eqs. 19 and 29); $_e\dot{S}\times\eta^*$: relative economic output power (Eq. 22); $_e\dot{S}\times\eta^*\times\kappa$: relative economic conductance (Eq. 28); σ: enthalpy/Gibbs energy ratio, heat dissipation–entropy production conversion factor (Eq. 14) calculated for excreted acids (aerobic, propionate-acetate; $\Delta_b H'_{H+} = -8$ kJ mol^{-1}) or for accumulated acids (others; $\Delta_b H'_{H+} = -25$ kJ mol^{-1}; see Table 1).

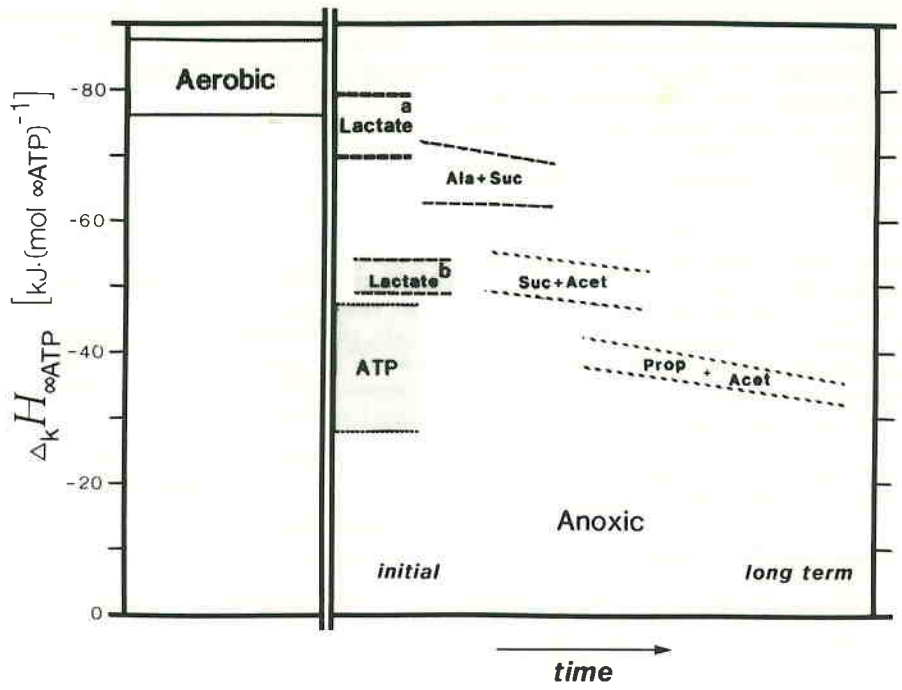

Fig. 8. The caloric equivalent of dissipative ATP turnover, $\Delta_k H_{\infty ATP}$, in aerobic and anoxic metabolism. The time course of decreasing catabolic heat dissipation, at constant rate of ATP consumption, is schematically represented for the aerobic-anoxic transition: Initially ergobolic hydrolysis of ATP contributes to the catabolic rate, and lactate may be formed from free glucose (a) or glycogen (b). Ala + Suc: Caloric ATP equivalent for succinate formation coupled to aspartate-alanine transamination (initially) or for alanine formed by ammonia fixation (later). Suc + Acet: Caloric ATP equivalent in the branched pathway from glycogen to succinate or with coupled acetate formation. Prop + Acet: As before, but with decarboxylation of succinate to propionate. In the last two boxes the decrease with time is due to decreasing buffer effects combined with an increasing fraction of excreted propionate and acetate (for details see Table 1).

'80; Zandee et al., '80). However, this result suggests an unexplained anoxic extra heat, because our analysis implies a greater decrease in heat output than ATP demand in the aerobic-anoxic transition, owing to the change of the enthalpy equivalent of anoxic ATP turnover (Fig. 8). Another implication of this thermodynamic analysis is that anoxic heat dissipation equal to 25% of the aerobic rate (Fig. 2) by the oligochaete *L. variegatus* corresponds to 50% of the aerobic rate of ATP turnover.

ENERGETIC EFFICIENCY AND RATE UNDER ENVIRONMENTAL AND PHYSIOLOGICAL ANOXIA

The above analysis indicates the importance of combined thermodynamic and biochemical considerations in interpreting rates of heat dissipation under aerobic and anoxic conditions. The caloric efficiency of catabolic pathways increases sequentially in the course of the aerobic-anoxic transition, and so does the biochemical ATP efficiency. The lowest ATP gain per substrate spent pertains to the lactate, octopine, strombine, and alanopine pathways ($\nu_{ATP/Glyc} = 3$) which predominate during burst activity or "anoxic recovery" (for reviews see di Prampero, '81; Livingstone, '82).

Why are the biochemically efficient pathways with the highest ATP yield not exploited under physiological hypoxia, when ATP demand is highest? Two arguments have been advanced to explain the restriction of the succinate-propionate-acetate pathways to environmental anoxia when ATP turnover rates are low (Zandee et al., '80). These authors suggest that compartmentalization and the occurrence of branchpoints prevent high rates. Both arguments lack theoretical foundation, because aerobic metabo-

lism is capable of high rates, despite its intercompartmental localization within cells, when oxygen supply is adequate. In addition, at a branch point, the competition of two enzymes for the same substrate would be expected to enhance its conversion.

What could solve the apparent paradox that the elevated demand for ATP during burst activity is met by biochemical pathways with the least efficient gain in ATP? Classical thermodynamics and calorimetric measurements, as applied above, do not relate to the specific problem of kinetics. Irreversible thermodynamics provides the theoretical framework to address this question. Detailed accounts of the expanding theory on biological energy conversion can be found in reviews by Caplan ('71), Rottenberg ('79), Stucki ('80), and Westerhoff and Van Dam ('79).

As a simplification, we assume linear relations between reaction rate and chemical affinity (Gibbs energy change of the coupled catabolic reaction)—i.e., between the flows and forces. This assumption is required for the application of linear irreversible thermodynamics, but must be tested by observation. High efficiencies (near-equilibrium situations of the coupled reactions) are approached during long-term anoxia, and hence linear relations may be expected. Lactate formation, however, is a highly irreversible and possibly nonlinear process (Table 2).

The direct calorimetric results also support for the applicability of linear relationships. The flow times the force is the power [mW] of the reaction, with the same dimension as heat dissipation. But in the context of kinetics, we are dealing with Gibbs energy changes rather than enthalpy changes. The resulting power is the dissipation function, ${}_i\dot{S}$, or rate of catabolic entropy production, ${}_k\dot{S}$, rather than ${}_k\dot{Q}$;

$$T \times \frac{\mathrm{d}_i S}{\mathrm{d}t} \equiv {}_i\dot{S} \tag{11}$$

$${}_k\dot{S} = \dot{N}_S \times -\Delta_k G_S \tag{12}$$

The dissipation function, ${}_k\dot{S}$, and the rate of heat dissipation, ${}_k\dot{Q}$, must not be confused (compare Eqs. 3 and 12). $\Delta_k G_S$ is the Gibbs energy per mole of substrate consumed in the catabolic half cycle reaction. For many anoxic pathways these two quantities can be very different. However, the constant rate of heat dissipation under long-term anoxia is a function of the constant rate of minimum entropy production (Table 2):

$$-{}_k\dot{Q} = \sigma \times {}_k\dot{S}, \tag{13}$$

$$\sigma = \frac{\Delta_k H_S}{\Delta_k G_S} = \frac{\Delta_k H_{\infty ATP}}{\Delta_k G_{\infty ATP}} \tag{14}$$

A constant rate of minimum entropy production characterizes linear systems in the steady state (Prigogine, '67) and hence is indicative, in combination with high thermodynamic efficiencies, of linear processes under long-term anoxia.

Economical output power and economical conductance

Complementary to the input power, ${}_k\dot{S}$, the output power, ${}_e\dot{S}$,

$${}_e\dot{S} = \dot{N}_{ATP} \times \Delta_e G_{ATP}, \tag{15}$$

is the rate of ATP production, $\dot{N}_{ATP}$, times the phosphorylation potential, $\Delta_e G_{ATP}$. Analogous to Eq. 9 we may express the catabolic input power on an ATP turnover basis:

$${}_k\dot{S} = \dot{N}_{\infty ATP} \times -\Delta_k G_{\infty ATP} \tag{16}$$

The efficiency is defined as the power ratio (Caplan, '71):

$$\eta = \frac{-{}_e\dot{S}}{{}_k\dot{S}} \tag{17}$$

In the steady state, the rate of ATP production, $\dot{N}_{ATP}$, equals the rate of ATP consumption and hence of ATP turnover, $\dot{N}_{\infty ATP}$. Therefore the catabolic efficiency of a fully coupled system, η^*, is defined by the force ratio (see Eqs. 15–17):

$$\eta^* = \frac{\Delta_e G_{ATP}}{\Delta_k G_{\infty ATP}} \tag{18}$$

Stucki ('80) proposed that mitochondrial systems maximize the "economical output power"—that is, maximum output power, ${}_e\dot{S}$, at minimum energy cost or at maximum efficiency, η. He derived the dependence of economical output power, ${}_e\dot{S} \times \eta$, on the ergobolic/catabolic force ratio and degree of

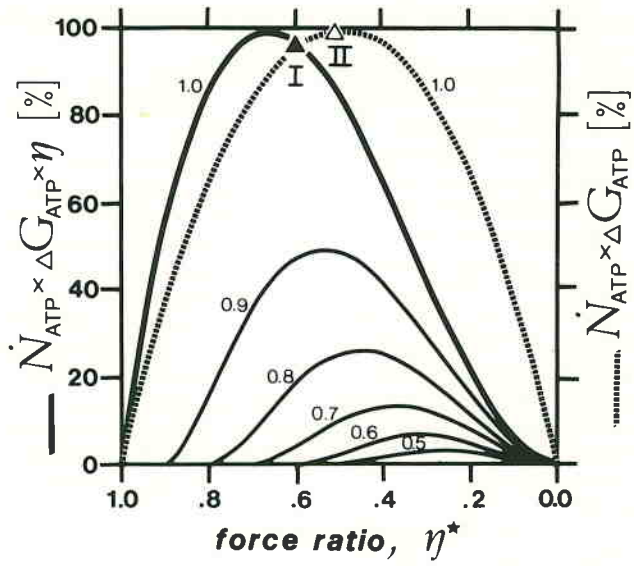

Fig. 9. The "economical output power," $\dot{N}_{ATP} \times -\Delta_e G_{ATP} \times \eta$ (solid lines), as a function of the ergobolic/catabolic force ratio, η^*, and of the coupling coefficient, x_{ATP} (shown by numbers), in a linear system (after Stucki, '80). For a fully coupled system the intercept of the normalized economic output power with the output power function, $\dot{N}_{ATP} \times -\Delta_e G_{ATP}$ (broken line) is shown. I and II denote the position of the glycolytic lactate pathway under initial and sustained physiological hypoxia, respectively (see Table 2; Eqs. 21 and 22).

coupling (Fig. 9). Maximizing the degree of coupling is seen to be the most effective mechanism for maximizing economical output power. Decoupling by 10% reduces the maximum economical output power by 50%. Therefore, substrate-level phosphorylation is an important mechanism of attaining, by stabilization of x_{ATP} at unity, an economical rate of ATP production. The lactate and the opine pathways fulfil this requirement, whereas phosphorylation reactions in the succinate and propionate pathway are susceptible to uncoupling to some extent (Holwerda and de Zwaan, '79; Schöttler, '77; Schroff and Schöttler, '77).

For a fully coupled system, the rate of ATP production can be described by the linear equation,

$$\dot{N}_{ATP} = L \times (\Delta_k G_{\infty ATP} - \Delta_e G_{ATP}), \quad (19)$$

where $L < 0$ is the phenomenological coefficient expressing the dependence of the flow, $\dot{N}_{ATP}$, on the net force $(\Delta_k G_{\infty ATP} - \Delta_e G_{ATP})$. By inserting Eq. 18 into 19

$$\dot{N}_{ATP} = L \times (1 - \eta^*) \times \Delta_k G_{\infty ATP} \quad (20)$$

and substituting the flow in Eq. 15 by Eq. 20 and the force by Eq. 18, we derive the output power function in the form

$${}_e\dot{S} = L \times \eta^* \times (1 - \eta^*) \times \Delta_k G_{\infty ATP}{}^2 \quad (21)$$

and the economical output power function as

$${}_e\dot{S} \times \eta^* = L \times \eta^{*2} \times (1 - \eta^*) \times \Delta_k G_{\infty ATP}{}^2. \quad (22)$$

Figure 9 shows that output power (maximum ATP production at a fixed phosphorylation potential) and thermodynamic efficiency cannot be maximized together. Therefore, under physiological hypoxia we might expect no maximization, but optimization of economy and power. This optimum is defined by the intercept of the two standardized functions; the corresponding characteristic efficiency is calculated from Eqs. 21 and 22 as $\eta = 16/27 = 0.593$. The actual efficiency combined with lactic acid production at the onset of burst activity was calculated at $\eta = 0.60$, shifting to 0.56 toward exhaustion. Clearly, diminu-

tion of efficiency cannot be avoided when achieving maximum rates.

The succinate-propionate-acetate pathways are, owing to their high force ratios, not capable of high rates of ATP production. In euryoxic animals, these highly efficient and more reversible pathways have evolved at the sacrifice of the potential of sustaining a high output power under environmental anoxia. Initially, propionate acccumulation is energetically inhibited at $\eta^* = 0.90$. In accordance with the observed delay in propionate production (Kluytmans et al., '78), the energetic inhibition is relaxed owing to a drop in the ATP/ADP ratio and a decrease in pH, despite the counteraction by increased propionate concentrations (Table 2). However, the efficiencies remain well above the characteristic force ratio for the maximum economical output power, $\eta = 0.667$ (Fig. 9). The latter cannot be a sufficient descriptor for the metabolic economy under environmental anoxia. Irrespective of thermodynamic efficiency, glycogen depletion as well as high concentrations of acid end products are detrimental per se, since 1) the substrate store is limited; 2) cellular acidification interferes with many other regulatory functions; and 3) excretion of end products is an energy loss unaccounted for in the expression of thermodynamic efficiency.

The concept of the economical output power function (Stucki, '80) must be expanded to obtain a meaningful expression for optimum catabolic economy under aerobic and anoxic conditions. This optimum function can be derived from the linear phenomenological equations for two coupled reactions:

$$\text{output flow: } J_1 = L_{11} X_1 + L_{12} X_2 \quad (23.1)$$

$$\text{input flow: } J_2 = L_{21} X_1 + L_{22} X_2 \quad (23.2)$$

In our specific case of fully coupled systems, $\eta = \eta^*$, these equations assume the form

$$\text{ergobolic flow: } \dot{N}_{ATP} = L \times -\Delta_e G_{ATP} + \frac{L}{\nu_{ATP/S}} \times \Delta_k G_S \quad (24.1)$$

$$\text{catabolic flow: } -\dot{N}_s = \frac{L}{\nu_{ATP/S}} \times -\Delta_e G_{ATP} + \frac{L}{\nu_{ATP/S^2}} \times \Delta_k G_S \quad (24.2)$$

Eq. 24.1 follows from Eq. 19 by inserting

$$\Delta_k G_{\infty ATP} = \frac{1}{\nu_{ATP/S}} \times \Delta_k G_S \quad (25)$$

$-\dot{N}_S = J_2$ is the rate of catabolic substrate depletion, and $L \times \nu_{ATP/S}{}^{-1} = L_{12} = L_{21}$ is the phenomenological cross coefficient showing that Onsager symmetry must be obeyed. The coefficients L_{11} and L_{22} relating the input and output flows to their respective conjugate force, are identified as L and $L \times \nu_{ATP/S}{}^{-2}$ in Eq. 24, expressing the system's conductance for phosphorylation and substrate consumption, respectively. In a situation of substrate limitation, a sufficient conductance for phosphorylation must be maintained at a minimum conductance for substrate depletion; in other words, the conductance ratio, κ:

$$\kappa = \frac{L_{11}}{L_{22}} \quad (26)$$

is to be maximized. For the fully coupled system (Eq. 24) the conductance ratio is simply related to the stoichiometric ATP-coupling coefficient frequently referred to as "biochemical efficiency":

$$\sqrt{\kappa} = \nu_{ATP/S} \quad (27)$$

The regulatory mechanisms controlling the conflict between economy and power in long-term anoxia can now be explained. Optimization involves a complex compromise: 1) the output rate, $\dot{N}_{ATP}$, and 2) the output force, $-\Delta_e G_{ATP}$, must ensure a sufficient output power or ergobolic dissipation function, ${}_e\dot{S}$, meeting the maintenance requirements. 3) The thermodynamic efficiency, η, is to be kept high, if the anoxic endurance strategy aims at a high reversibility of metabolic processes for the restoration of the glycogen pool during the aerobic recovery period. 4) A high conductance ratio, κ, provides the most effective use of the substrate store and minimizes the load on the end-product sink (accumulation of acids) or minimizes energy loss in excretion. These arguments are summarized in the *economical conductance* function:

$${}_e\dot{S} \times \eta \times \kappa = L \times \eta^{*2} \times (1 - \eta^*) \times \Delta_k G_S{}^2. \quad (28)$$

This expression for economical conductance follows from Eqs. 22 and 27 with $\Delta_k G_{\infty ATP} \times \nu_{ATP/S}$ substituted according to Eq. 25. While succinate production in combination with acetate has the highest economical conductance initially (I, Table 2B) the propionate-acetate pathway is superior to any other in terms of economical conductance during long-term anoxia (II, Table 2B). In the changing cellular environment, there is a strict relation between rate, economy, and the patterns of primary and secondary anoxic end-product formation.

Heat dissipation and dissipation function

The foregoing analysis has provided a rationale for the imposed divergence of biochemical optimum functions sustaining physiological and environmental anoxibiosis. The decrease of heat dissipation observed in long-term anoxibiosis occurs because the steady-state rate of anoxic ATP turnover is constrained by the decreased catabolic net force (Fig. 10) and because of the reduction in the caloric equivalent of anoxic ATP turnover (Figs. 8, 10).

To illustrate the energetic control mechanisms operative in the succession of multiple endproduct formation, Eq. 20 is rewritten for contrasting the ATP turnover rate, the catabolic dissipation function (entropy production), and the catabolic heat dissipation (see Eqs. 16 and 13):

$$\dot{N}_{\infty ATP} = L \times (1 - \eta^*) \times \Delta_k G_{\infty ATP} \quad (29)$$

$${}_k\dot{S} = -L \times (1 - \eta^*) \times \Delta_k G_{\infty ATP}{}^2 \quad (30)$$

$${}_k\dot{Q} = L \times (1 - \eta^*) \times \Delta_k G_{\infty ATP}{}^2 \times \sigma \quad (31)$$

L is considered invariant with time and independent of the pathway (linearity assumption), while all other variables are a function of the catabolic process and of the changing cellular environment (Table 2A). The anoxic rates are expressed as a percentage of the

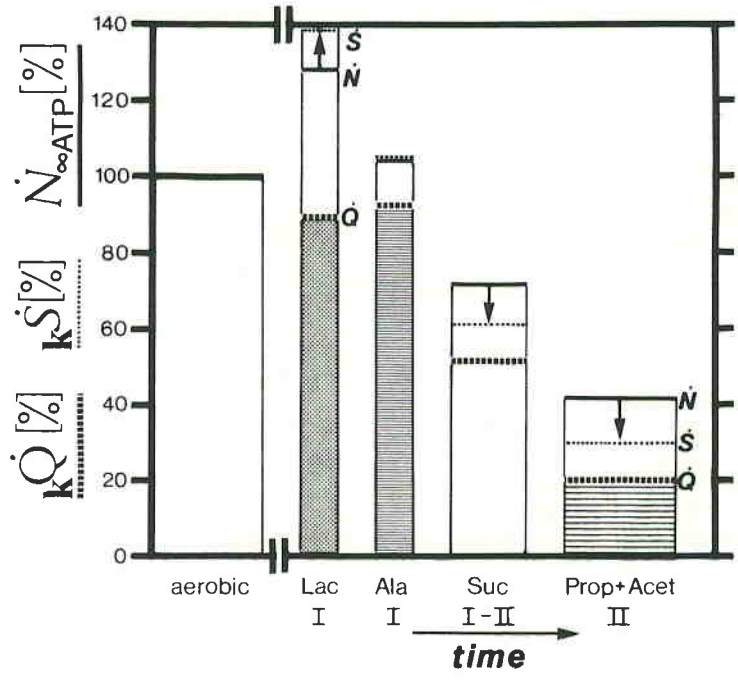

Fig. 10. Relative aerobic and anoxic rates of heat dissipation, ${}_k\dot{Q}$ (shaded columns, broken lines), catabolic dissipation function or entropy production, ${}_k\dot{S}$ (dotted lines), and rate of ATP turnover, $\dot{N}_{\infty ATP}$ (solid lines), calculated from Eqs. 29, 30, and 31, as a function of the anoxic pathway and cellular conditions changing with time (I, II, see Table 2 and Fig. 8).

normoxic rates (Table 2B). While the lactate pathway reveals a potentially higher ATP turnover rate than aerobic respiration, ATP turnover associated with succinate, propionate, and acetate production theoretically decreases by 30–60%. The relative changes of the catabolic dissipation function show an amplification of the trends of ATP turnover (Fig. 10). This is due to the proportionality between rates and Gibbs energy potentials of dissipative ATP turnover (Eqs. 29 and 30). In contrast to the strictly divergent relation between turnover rate and catabolic dissipation function, there is no phenomenological interdependence between heat dissipation and entropy production (the dissipation function). The proportionality is not related to the kinetics, but is a function of the specific catabolic reactions and side reactions (Fig. 8). From Eq. 31 and with thermodynamic variables calculated for realistic cellular and ecological conditions, the relative rate of heat dissipation is predicted to scale down to 20% in long-term anoxibiosis, when only propionate and acetate are produced and excreted (Fig. 10). The experimentally observed decline of ATP turnover and heat dissipation agree with our thermodynamic predictions.

Figures 9 and 10 illustrate the necessity for selecting a thermodynamically and biochemically inefficient pathway for generating a high output power at optimum efficiency during burst activity. The lactate pathway is well suited for this purpose. The "opine" pathways probably fall into the same category (see Fields and Quinn, '81). The succinate-propionate-acetate pathways exhibit the highest *economical conductance* under the cellular conditions predominating in euryoxic animals under anoxia (Table 2). This suggests that *economical conductance* may have been selected for long-term anoxibiosis because economy was more vitally important than power. A high rate of ATP demand is promoted by incurring a necessarily high irreversible energy loss, while an efficient ATP gain prevents the development of a high output power, but supplies more ATP in the long run, as required for anoxic survival. Maximum rate and maximum efficiency are mutually exclusive.

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