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Calorimetry and thermodynamics of living systems☆

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Abstract

Calorimetry of living systems and classical thermodynamics developed in parallel, from Lavoisier's early ice calorimeter experiments on guinea pigs, followed by Dubrunfaut's macrocalorimetric research of fermentation processes and Atwater-Rosa's whole-body calorimetry on humans and domestic animals, to the introduction of the famous Tian-Calvet instrument that found entrance into so many different fields of biology.

In this work, six examples of living-system calorimetry and thermodynamics are presented. These are: (i) glycolytic oscillations far off the thermodynamic equilibrium; (ii) growth and energy balances in fermenting and respiring yeast cultures; (iii) direct and indirect calorimetric monitoring of electrically stimulated reptile metabolism; (iv) biologic and climatic factors influencing the temperature constancy and distribution in the mound of a wood ant colony as an example of a complex ecological system; (v) energetic considerations on the clustering of European honeybees in winter as a means to save energy and stored food as well as for their Japanese counterparts in defending against hornet predators; and (vi) energetic and evolutionary aspects of the mass specific entropy production rate, the so-called bound dissipation or psiu-function.

The examples presented here are just a very personal selection of living systems from a broad spectrum at all levels of complexity. Common for all of them is that they were investigated calorimetrically on the background of classical and irreversible thermodynamics.

Keywords: Animals; Direct and indirect calorimetry; Ecological systems; Irreversible thermodynamics; Microorganisms; Oscillations

1. Introduction

It is always astonishing to realize how intimately early calorimetry and thermodynamics were coupled with living systems. This might be partly due to the fact that fire was taken as a symbol of life for many centuries or even millennia and the end of its burning as synonym for death. Already Leonardo da Vinci observed in the famous grotta de cane near Naples that animals (dogs) cannot survive in an atmosphere where a flame goes out. Three centuries later, Joseph Priestley showed experimentally that in a closed air volume a fire would extinguish and a mouse would die, an experiment that was repeated by AntoiNe Laurent Lavoisier. He placed a burning candle and a bird together in a glass jar and observed that they both expired at the same time. His famous La vie est donc une combustion can be cited in a more comprehensive form as "Life is a slow combustion maintained through respiration. Animal bodies are composed of combustible elements. Food replaces the loss of body substances resulting from the combustion of some of the matters present in the body" [1]. Although both Priestley and Lavoisier were still deeply embedded in the idea of phlogiston (the material component of all

☆ In memoriam Prof. Alexander I. Zotin (1926–2000), Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow, who made so many essential contributions to calorimetry and biological thermodynamics.

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combustible material and base metals that escapes during burning), their detection of oxygen as the essential gas component in air opened the way to modern chemistry and to bioenergetics as well.

Direct calorimetry of living systems supposedly started in 1777 with Adair Crawford's investigation of guinea pigs in a calorimeter similar to the one that was used by Priestley for the combustion of hydrogen. Crawford reported about "the quantities of heat communicated to 31 pounds seven ounces of water that 100 ounce measures of pure air by the combustion of wax imparted 21 degrees, by the combustion of charcoal 19.3 degrees, by the respiration of a Guinea pig 17.3 degrees" [2] and that the production of heat was proportional to the change in the air. The various investigations of Lavoisier and Pierre Simon de LaPlace (1780) with their ice calorimeter followed, including the one with a guinea pig that melted 13 oz (370 g) of ice within 10 h, thus dissipating a mean metabolic power of 3.4 W. The question remained open as to whether all heat originated from the combustion of carbon or whether other sources also contributed (the famous 1822 prize-study of the Paris Academy of Sciences *The Determination of the Source of Animal Heat*). Césav-Mansuète Despretz (1824) and Pierre Louis Dulong (1841) participated with very similar calorimeters, monitored the heat production and the respiration of guinea pigs, and found a significant discrepancy between both results. Their conclusion was that the combustion of carbon and hydrogen alone could not be responsible for the animal heat [3,4].

Thermodynamics entered the game when Lavoisier formulated his law of the conservation of mass and observed that the weight of a substance increased during oxidation but did not decrease following the ideas of phlogiston. Living systems were—presumably—included implicitly. But the two main steps were the establishment of the law of conservation of energy by Robert Mayer (1842)—the First Law of Thermodynamics—and its specialisation as the constant sum of reaction heats by GerMain Henri Hess (1840). Max Rubner's experiments with dogs in a respiration calorimeter during the 1890 showed that Hess' law was applicable not only in chemistry, but also in biology and that there were no principal thermodynamic differences between inanimate and living systems.

A first step away from the well-known small animals like guinea pigs and rabbits to the rather unknown microbial systems was done by Augustin Pierre Dubrunfaut, a French scientist in the middle of the 19th century, who was strongly engaged in the refining of sugar, the French sugar-beet industry, and alcoholic distillation [5]. He—the father of modern macrocalorimetry—made one essential experiment in 1856 to determine the heat production accompanying alcoholic fermentation. Twenty-one thousand four-hundred litres of molasses solution with 2559 kg of sugar were kept in a huge oak fermentation vat of 3 m height and diameter. During 4 days of fermentation the broth temperature rose from 23.7 °C to a mean 33.7 °C while the ambient temperature fluctuated between 12 °C and 16 °C. Dubrunfaut followed the temperature decrease at the end of the experiment and calculated heat losses due to radiation and convection by means of Newton's Law of Cooling. Moreover, he took evaporation, heat storage in the oak wood and the transition of carbon dioxide from the solution into the atmosphere into account so that he finally arrived at a heat of fermentation of 94.9 kJ mol^{-1} glucose equivalent. This is rather low in the light of the 138.6 kJ mol^{-1} predicted by the Gay–Lussac equation for the fermentation of glucose to ethanol and carbon dioxide. Battley showed in his well-known book *Energetics of Microbial Growth* that storage of sugar, assimilation into biomass and growth of yeast during this experiment are partly responsible for this difference [6] (see also Section 3 for energy balances during yeast growth).

Classical thermodynamics tacitly developed in closed systems. It was within this framework that constancy of mass and energy, thermal equilibria, steady increase of entropy and the *heat death of the universe* (Rudolf Clausius) were considered. Also, an intelligent creature like Maxwell's demon was born and Felix Auerbach could talk about *The World Mistress and her Shade* (energy & entropy) and call living systems *deceivers in the game of entropy* [7]. Maxwell's demon is meanwhile exorcized by Leo Szilard [8,9] showing that information is necessary to get rid of entropy (or to gain negentropy [10,11]), but still to the present time such friendly brownies are supposed to be active in biological structures like membranes and enzyme complexes or even in atomic reactors [12].

Living entities are—quasi by definition—thermodynamically open systems in a continuous exchange of matter, energy, entropy and information with their surroundings and inevitably have to die when they are isolated to closed systems approaching a thermodynamic equilibrium. Living organisms are more or less far off such equilibria, may develop steady states and are ruled by irreversible processes where entropy dissipation plays an important role.

In the present paper, own calorimetric investigations, from more simple oscillating (bio)chemical reactions, to microorganisms and insects, up to larger animals and complex social organizations (ant hills and bee clusters) shall be discussed in connection with biological thermodynamics. A historical order of the given examples depending on the appearance of special thermodynamic laws or aspects is not intended.

2. Oscillating reactions

In systems far from the thermodynamic equilibrium unusual phenomena like limit cycles, instabilities, chaos, bifurcations, dissipative structures and oscillations may happen that attract scientists from the theoretical as well as the experimental side. In particular, oscillations in (bio)chemical reactions and biological processes fascinated researches from different fields. Well-known biological phenomena like pattern formation, circadian rhythms, intestine peristalsis or fibrillation of heart tissue suddenly found a common explanation.

Spectroscopy, polarography, manometry, chromatography and ion-selective electrodes were used for the investigations of oscillating reactions, and only in a few cases also calorimetry, although the last one is the only technique that renders not just a picture of the momentary status of the system but also about the energy flow through it. Adiabatic as well as isoperibolic instruments can be used to monitor the heat production rate in such reactions, at best in combination with other techniques like potentiometry or spectroscopy. Although many pure chemical oscillations like the renowned Belousov-Zhabotinskii, the Briggs-Rauscher and several ABA reactions were investigated calorimetrically in the author's group [13], only glycolytic oscillations [14–16] will be dealt with because of the living systems in the title.

Organisms may degrade glucose or other sugars anaerobically to obtain the energy rich molecule adenosine triphosphate (ATP). A special type of this glycolysis is the alcoholic fermentation found in yeast cells (see Section 3). During this type of metabolism oscillations can occur in different metabolites, especially in the NAD^+/NADH system, which is usually monitored photometrically in the near UV range, but also calorimetrically [13–16]. Glycolytic oscillations appear not only in yeast, but also in a manifold of biological systems like bovine heart extracts, tumour cells or muscles of flies.

All experiments were performed in a 1.2 ml calorimeter (Triflux, Thermanalyse, Grenoble/France) that could be equipped with a light guide/photo diode system to monitor heat production rate and UV absorption simultaneously [17]. Due to the thermal inertia of the calorimetric signal it has to be desmeared to show true coincidences or phase shifts between the extrema of the two signals.

Glycolytic oscillations are best seen in cytoplasmic extracts [14,16] but not so much in whole cells [18] and occur only in special windows of substrate flow rates which lie in this case between 10 mmol l^{-1} h^{-1} and 160 mmol l^{-1} h^{-1} [15]. They can be obtained by a technique of glucose injection or by using the disaccharide trehalose as a sugar source. It is split by the enzyme trehalase into two glucose molecules at a rate that fits well into the window. A corresponding experiment is shown in Fig. 1 for the heat flow: a first oscillatory period starting at a high turnover rate and with large amplitudes that are damped out to a short (second) steady state period without oscillations, but still considerable energy flow. The experiment ends after a transition phase that reflects some kind of a Michaelis–Menten kinetics in a fourth period with nearly no heat production—the energy source trehalose is used up. The slope of Fig. 1 confirms the statement that phenomena like bifurcations, oscillations or even chaos (often observed in unsuccessful experiments of this kind) are only seen far off thermal equilibrium and vanish when it is approached.

The area under the graph corresponds to the reaction heat and is proportional to the amount of added trehalose. One obtains a figure of $-179\,kJ\,mol^{-1}$ trehalose or $-89.5\,kJ\,mol^{-1}$ glucose that allows for a transformation of the energy into substrate concentration values. Thus, the heat flow picture is changed into

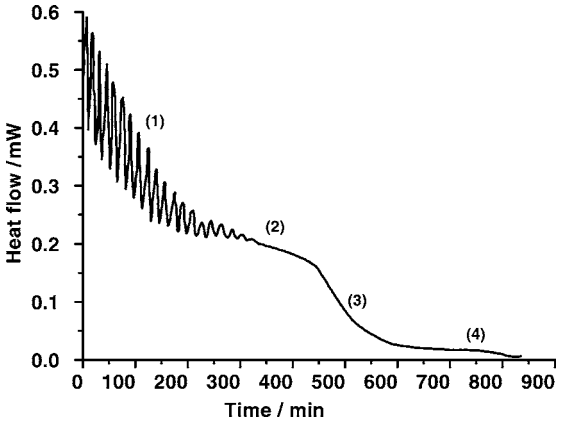

Fig. 1. Heat-flow curve of an oscillating extract from baker's yeast *S. cerevisiae* with the typical four different phases: (1) oscillatory window; (2) steady state; (3) transition period; (4) exhaustion of the substrate trehalose (adapted from [13]).

a substrate flow picture that enables a continuous kinetic interpretation of the experiment—only possible with calorimetry and with no other standard technique. It shows that the transitions between the three periods always occur at the same substrate concentration independent of their initial values.

As most other investigations on glycolytic oscillations were run with NAD^+/NADH monitoring, the light guide system mentioned above [17] was applied rendering pictures as in Fig. 2. The first striking result

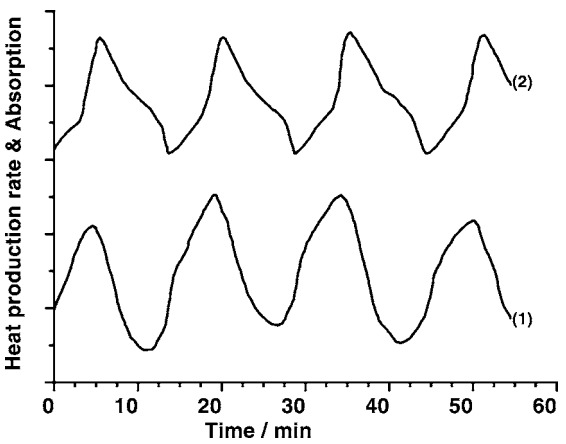

Fig. 2. Enlarged oscillatory period of a yeast extract (*S. cerevisiae*) with the simultaneous registration of (1) the heat flow and (2) the $NAD/NADH^+$ absorption, both given without units just to show the structures in the slopes (adapted from [13]).

is that there are no sinusoidal oscillations as supposed from Fig. 1 but well structured traces with at least four distinct phases in one period. They are more pronounced in the absorption curve but are nearly as well defined when the calorimetric signal is desmeared for its thermal inertia.

3. Microorganisms

Since the times of Dubrunfaut and Rubner, microbial investigations played an important role in classical as well as modern calorimetry and stimulated a number of highly sophisticated instrumental designs to offer appropriate growth or existence conditions for microorganisms. A prototype of this development is the LKB flow calorimeter constructed by Wadsö and Monk in the end of the sixties [19,20]. In parallel, many thermodynamic conclusions were drawn from such experiments, primarily concerning the First Law, then the Second Law also, and of course many new ideas in kinetics. The following example touches some of them.

Bakers yeast *Saccharomyces cerevisiae* was grown at 30 °C in a liquid glucose medium under intensive stirring in an external 1 l fermentor in combination with an LKB-10700-1 flow microcalorimeter [21]. To overcome the usual problem of oxygen depletion in the flow line from the fermentor to the calorimeter, air bubbles were introduced into the flow in a ratio of 1:1 with the medium. It could be proved that the yeast metabolism ran under completely aerobic conditions. Nevertheless, the power–time curve of growth in a pure glucose medium followed a highly structured slope with several maxima (Fig. 3). Simultaneous determinations of medium composition, oxygen consumption, energy stored in biomass (bomb calorimetry) and mass specific growth rate show that the first exponential phase includes aerobic fermentation of glucose to ethanol and that the cells respire the accumulated ethanol in a second one (glucose–ethanol diauxy). Two other structures are visible in the slope: an oscillatory transition from glucose fermentation to ethanol respiration and the aerobic consumption of a further metabolic product at the end of growth. Energy and carbon balances (Fig. 4) rendered an enthalpy carbon quotient of 445 kJ mol^{-1} C that fits to lactate as well as to acetate. But as oxygen consumption

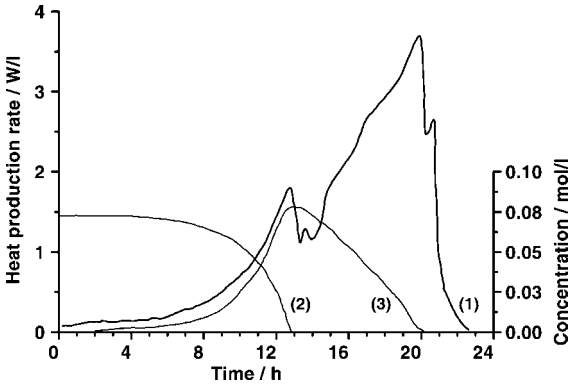

Fig. 3. Power–time curve of (1) a yeast batch culture (*S. cerevisiae*) from a combination of a flow calorimeter and an external fermentor, together with the simultaneously determined curves for (2) glucose and (3) ethanol concentration (adapted from [21]).

continues in this last phase, lactate drops out and acetate remains as last energy and carbon source. The steep declines after the three maxima mirror the sensitive changes in enzymatic rates at low concentrations in the Michaelis–Menten kinetics [23].

Fig. 4 shows a time dependent energy balance for yeast growth in a liquid medium with glucose as the sole energy and carbon source. This balance is presented in relative units as it is similar for all initial glucose concentrations. And it is qualitatively the same for a carbon balance when the sector heat is exchanged

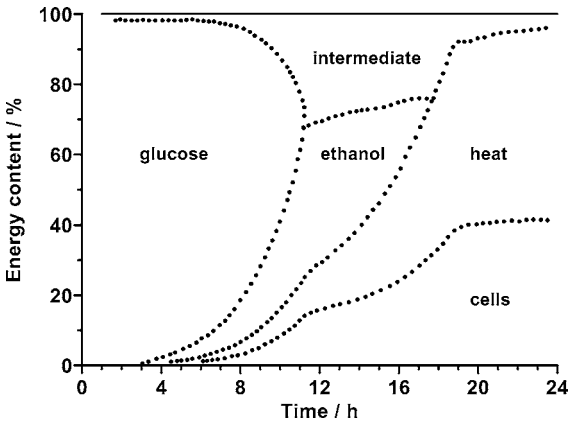

Fig. 4. Time course of the energy balance for calorimetrically determined yeast growth (*S. cerevisiae*) in a calorimeter–fermentor combination as for Fig. 3. The different energy contents are given in percent of the total amount of energy (adapted from [21]).

for a carbon dioxide sector. All balances are equable except for the at first unexpected intermediate metabolite which turned out to be acetate (see above) and they thus obey the First Law. But the result underlines that 40% of the ethanol formed in the first phase of growth is immediately oxidized to acetate under the chosen experimental conditions and that only 10% of the dissipated heat is a by-product of glucose fermentation.

The oxycaloric coefficient of growth is expected to be $0.46\,\mathrm{kJ\,mmol^{-1}}$ O_2 for glucose. But it is significantly higher (up to $0.7\,\mathrm{kJ\,mmol^{-1}}$ O_2) in the first period due to a pronounced contribution of fermentative reactions that do not consume oxygen but dissipate heat. Later on the coefficient drops below the theoretical value because an important part of energy (45% of the initial glucose energy) is stored in the biomass.

These complex microbiologic investigations were used for further thermodynamic calculations [24,25]. As will be shown in Section 7 of this paper, the volume specific dissipation function $\psi = T/V\,\mathrm{d}S/\mathrm{d}t$ may be split in two parts, the bound dissipation ψ_u and the external dissipation function ψ_d: $\psi = \psi_\mathrm{u} + \psi_\mathrm{d}$ [26]. ψ_d originates from the external dissipation, ψ_u represents the bound dissipation remaining inside the system. Most global dissipation processes in living systems are driven by energy-yielding catabolism, by glycolysis and/or respiration that can be monitored by the usual manometric or Warburg techniques so that the total ψ is well-known. External dissipation ψ_d is a result of heat production and liberation during metabolism which is observed by calorimetry. Only the bound psiu-function ψ_u has no direct counterpart but it is easily determined as the difference between the two first terms. Following the thermodynamic rules of open systems far off the thermal equilibrium, ψ_u should be high in early stages of an organism and decrease towards a minimum or even to zero during the following development.

In a broader sense, a growing microbial culture may be considered as an organism so that the above ideas are applicable to it. Fig. 5 presents the time course of yeast growth monitored as total metabolism (glucose fermentation followed by ethanol and acetate respiration) and heat production rate, both in mass specific units. Their difference renders the psiu-function that shows the expected three maxima and the three subsequent declines. The first maximum corresponds to the transition from the microbial lag phase to the

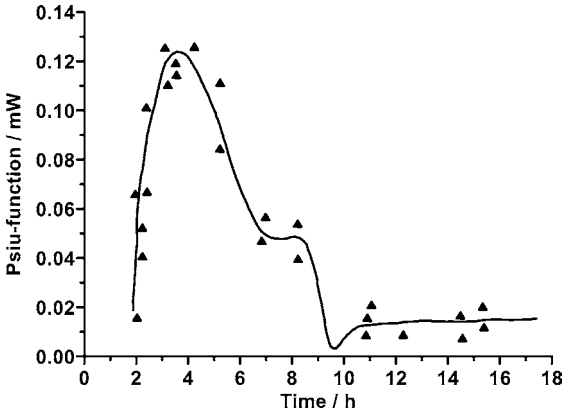

Fig. 5. Bound dissipation function (psiu-function; solid line) and the mass specific growth rate (points) during the development of a yeast culture (*S. cerevisiae*) under anaerobic conditions (adapted from [24]).

exponential phase when the young organism is in a highly active state binding a considerable part of the metabolic energy for synthetic processes. In the exponential period the growth reaches a balanced equilibrium with a constant rate and a minimal dissipation: the psiu-function tends towards zero. The new energy source ethanol produces again a young organism, which is in the beginning far off the equilibrium with a second maximum of the psiu-function. The oscillatory transition period may be considered as a third young organism in the course of microbial development.

It is worthwhile to discuss these results in some more details. The first growth phase of the microbial culture (one of the three substrates) is the lag phase, an intracellularly highly active time with enzymatic adaptation to the new substrate and regulation processes for cell multiplication. The following exponential phase exhibits a high energy turnover, but in a balanced state as long as the external conditions allow for it. It may endure for days, weeks or even longer in so-called chemostat cultures [22] that are not considered here. This is a period without macroscopic changes, without aging of the organism; the culture is in equilibrium and biochemical processes are near to a thermodynamic steady state. At this time, the psiu-function is constant and at a very low level. The third or stationary phase of culture growth is defined by a lack of substrate or by unfavourable external conditions (e.g. pH value, blocking concentration of a metabolic product, high cell density) so that the culture retires to a resting state without net growth and with a low metabolic rate. An amelioration of the growth conditions would create a new organism and drive the psiu-function to a further maximum. In total, the stationary phase is an artificial state outside the normal life cycle so that the psiu-function as the bound entropy production during growth and development should not be applied to this period [22].

4. Direct and indirect calorimetry of small animals

Not only microorganisms are able to switch between aerobic and anaerobic metabolism but also animals on all levels of complexity. While respiration in tissues is dependent on the rather small amount of oxygen dissolved in them and mainly on the transport of oxygen to them, anaerobic fermentation utilizes substrates present in those places where the energy is urgently needed. Reptiles are good examples for the change to an anaerobic metabolism in moments of high activity, e.g. when hunting or escaping. As their trunk muscles are involved in locomotion, it is more or less impossible for lizards and snakes to breathe and ventilate their lungs during bursts of movement. In such stress situations muscle glycogen/glucose is fermented to lactate with ATP production. As no gaseous components are involved in this step, anaerobic glycolysis is not open to manometry and thus to the usual indirect calorimetry. The only way to estimate the anaerobic contribution lies in the determination of the lactic acid concentration in blood or tissue cells, which often means the sacrifice of the experimental animal [27]. Direct calorimetry, however, measures heat production from whatever reaction including fermentation.

With this in mind, a combination of direct and indirect calorimetry was run on six species of lizards (16 animals) and three species of snakes (12 animals), among them some Milos wall lizards (*Podarcis milensis*) and Aesculapian snakes (*Elaphe longissima*) [28–30]. The weight of the lizards varied from 2 g to 8 g, that of snakes from 7 g to 14 g. The prolonged periods of rest shown in the power–time curves (see below) demonstrate that the 100 ml calorimeter vessel was comfortable and without stress for the reptiles. These open, or hermetically sealed vessels of an isoperibolic

Calvet microcalorimeter (SETARAM, Lyon/France) were equipped with a polarographic oxygen sensor on top outside the area of heat flow measurement and a two-electrode system to stimulate the animals [28]. The electrodes were connected to a generator of harmless 30 V pulses, and an ohmmeter to determine the moment when the experimental animal was in good contact with the two electrodes. To facilitate the voltage application and to avoid unnecessary high voltages, the animals were smeared with an electrode cream as used for EEG or ECG. At the instant of contact, the electrode resistance dropped from megAohm figures to some 10 kΩ before the voltage was switched on for 30 s. A burst of activity followed visibly at first as a strong increase in the heat production rate of the animal and then as a deviation in the beforehand steady decrease of oxygen tension. In general, the calorimetric signal as a derivative figure (a rate) renders by far more information than the integrative oxygen concentration slope.

Power–time curves of lizards are generated by short activity pulses and longer periods of rest, even more than those of snakes (Fig. 6). These low levels of heat output may be ascribed to the basal metabolism of the animals. The locomotor bursts that are so clearly seen in the calorimetric curve are not emphasized in the oxygen curve, partly due to its integrative character, but more to the fact that they are powered by the short-term fermentation of glycogen to lactate. The picture changes when the animals are electrically stimulated (Fig. 7) after a more or less constant pre-period. The area of the pulse corresponds to a heat output of 22.9 J, the corresponding deviation in the oxygen slope to 1.1 ml oxygen, rendering an oxycaloric equivalent of 20.8 J ml^{-1} oxygen.

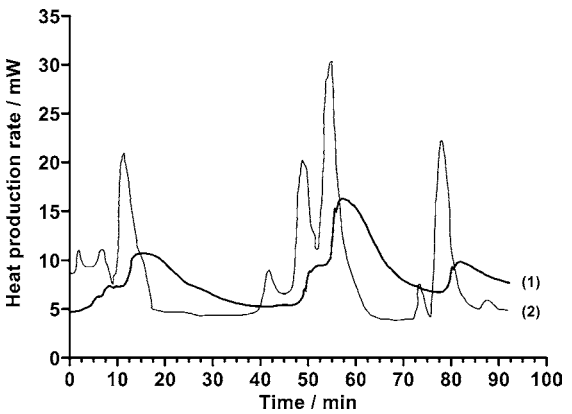

Fig. 6. Power–time curve of (1) a Milan wall lizard (*P. milensis*) of 3.7 g at 25 °C in a calorimeter vessel open to air. The periods of activity and rest are evident. The transformed signal ((2); "desmeared" for the thermal inertia of the calorimeter by means of the Tian equation [2]) is also given to show the burst like character of the stimulated metabolism.

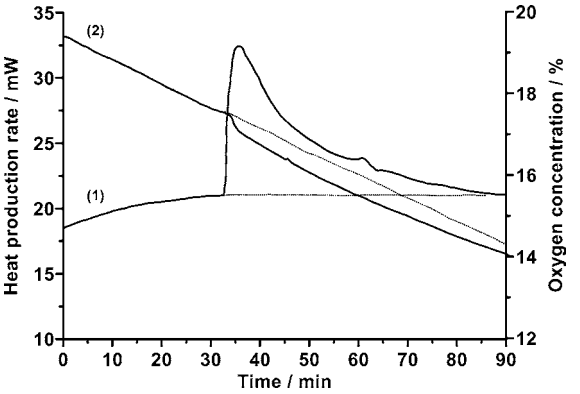

Fig. 7. Power–time curve (1) and oxygen consumption trace (2) of a 3.7 g Milan wall lizard (*P. milensis*) during pre-, stimulation- (30 V, 30 s) and post-period at 30 °C.

Power–time curves of lizards and snakes were evaluated for their mean, long-term metabolic turnover, including both periods of activity and of rest, as well as the maximum and minimum values. The mass specific mean heat production rates at 25 °C amounted to 1.8 mW g^{-1} for lizards and to 0.6 mW g^{-1} for snakes. The maximum values came to 3.4 mW g^{-1} and 0.9 mW g^{-1}, the minimum figures to 1.1 mW g^{-1} and 0.5 mW g^{-1}, respectively. The resting metabolism (p_{min}) contributes 74% to the mean heat production (p_{mean}) for snakes and only 59% for lizards, showing that the snakes are significantly less active than the lizards. In total, the direct calorimetric data are in good agreement with indirect data from the literature when they are transformed to the same experimental temperature. But the observed increase in metabolism due to the short electric shocks cannot be compared with the highly increased oxygen consumption of other lizards from touching hind limbs and tail and with the exhaustive prodding stimulation of 30 s or 5 min that two African lacertid lizards experienced [31,32]. Exercised animals were killed immediately after the test, homogenized and the lactate content in

their body was determined. The oxygen consumption rate in the first 30 s increased by more than a factor of 10. An intensive lactate production took place during the 5 min prodding rendering a total energy turnover of about 40 times that of the resting metabolism with about 70% contribution from the lactic fermentation [31].

5. An ant hill as an ecological system

Living systems are not only organisms sensu strictu but may also be aggregations of many living entities of one species (e.g. a bee hive or a termite mound) or of several different ones, e.g. ant hill). Ecological systems are rather complex and hard to analyse as especially in this case *the whole is more than the sum of the individual components*. Here, the nest of the red wood ant, *Formica polyctena* Foerst., is chosen as an example for such a complex living system. It was investigated by means of several types of calorimetry (adiabatic, isoperibolic, combustion) and by manometric, thermometric and meteorologic methods together with mere biological observations. The aim was not only to find the source of the nest heat used by the ants for internal nest thermoregulation, but also to differentiate between biotic and abiotic internal and external factors, to determine the heat flow through the ant colony and to establish heat balances.

Several ant hills in a mixed oak–pine forest of Berlin were included in these investigations for a complete season (March–October) with pronounced activities of ants (and researchers) during June and July. A typical hill with a diameter of about 100 cm, a height of 30 cm (and a depth of 50 cm into the ground) was chosen with the following description [33]. Its volume was about 570 l, the fresh weight 82 kg and it was populated by 110,000 ants (1.1 kg). Surface temperatures of the hill fluctuated between 16 °C and 47 °C, while the center kept its value constant at about 26 ± 0.3 °C. This was mainly due to two facts, namely that the intensive sun radiation upon the inclined hill sides enters only a very thin surface layer and that the ant workers knew how to regulate the center temperature by forced ventilation.

It was a well-accepted opinion till the fourth quarter of the last century that all biogenic heat in an ant hill originates from the adult ant workers. Our own experiences with such systems raised severe doubts as to the sterility inside the mound and resulted in calorimetric and manometric investigations on adult ants, pupae and the nest material they live in. The general assumption of a high mass specific heat production rate of workers was correct: 2.6 mW g^{-1} fresh weight, significantly lower for pupae 0.8 mW g^{-1} and only 0.18 mW g^{-1} or 0.32 mW g^{-1} for nest material from the periphery or the centre of the hill (all values at 20 °C). But taking into account that our model mound had a mass of 82.3 kg and a population of 1.1 kg ants and only 0.04 kg pupae, the total heat dissipation changed to 2.86, 0.03 and 16.46 W, for the three categories, respectively. This means that 14.8% of the total heat output of the hill are contributed by the adult ants, less than 0.01% by the pupae and 85.1% by the nest material. The doubts were apparently justified.

The model hill was analysed as a one-centre-two-shells system with the temperature maximum and thus the highest heat production rate in the centre near to the brood. The peripheral shell amounted to 52 kg, and the intermediate to 27 kg. The 5 kg centre had the largest contribution to the biogenic heat production due to both its elevated temperature and its rich substrate and intensive microbial activity. One may assume that more than 50% of the heat balance originate from the centre. Adiabatic calorimetry showed self-heating processes in the nest material leading to final temperatures above 80 °C (that are never observed in an ant hill) indicating that mesophilic, thermotolerant and thermophilic microorganisms became consecutively active in such laboratory experiments. The final temperatures were dependent on the season, with highest values in June and July and minima during winter. The total heat output in such experiments followed a similar time course [34].

In addition to these lab-bound experiments, in situ investigations were carried out in a Berlin forest [34,35]. An ant hill of approximately the same size as the above mentioned model system was monitored throughout the whole ant season by 12 thermocouples: 1 for air, 1 for soil, and 10 for different positions in the nest between the surface and the centre. The data were continuously recorded and completed by the local weather service. Direct radiation from the sun in various wavelength ranges and back radiation from the hill were measured during a few days of selected weather situations. Further climatic factors such as wind in different levels above ground, rain,

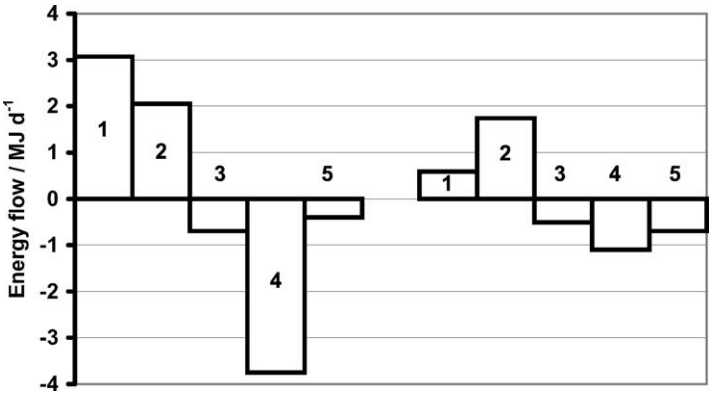

Fig. 8. Heat balances of an ant hill for 2 days with sunny (left) and rainy weather (right). (1) Heat flow due to radiation; (2) biogenic heat production; (3) heat exchange with air; (4) heat exchange by evaporation; (5) heat conduction to the soil (adapted from [36]).

and relative humidity were taken into consideration when energy balances between day and night or fine and rainy days were established. Fig. 8 compares such weather differences. It becomes clear from the bars that the biogenic heat production remains a rather stable component while radiation and evaporation diminish dramatically. Heat exchange with air and soil are small and without essential fluctuations. Fig. 8 underlines how important the biological contribution is to the micro-climate in the ant hill and within it to that of the microbial decomposition of the nest material.

6. Honeybee clusters

There were numerous heat production investigations of honeybees by means of indirect calorimetry (manometry, thermometry plus cooling laws), and also some by means of direct calorimetry that showed high to very high mass specific metabolic turnover rates. But to assume that the total heat output of a group of bees or even a colony is linearly correlated with the number of insects is erroneous. Nearly an inverse relation holds good in smaller groups [37]. Bees as social insects become nervous or hyper-active when isolated and calm down when they are together with other members of their hive or even more when they can take care of the queen or the brood [37]. An isolated bee at 20 °C exhibits a mass specific heat production rate of 280 mW g^{-1} wet weight but this enormous rate decreases hyperbolically (regression coefficient $R^2 = 0.985$) with increasing group size to 5.2% of this value for 18 bees. Single-bee values show the metabolism of active, sometimes even flying bees. It is known from a variety of studies that honeybees have enormously large metabolic rates during flight. Their flight muscles exhibit the highest energy turnover in the organismic world. This means that single-bee values are artificial and should be considered with care when energy balances are established for honeybee colonies or clusters.

The above mentioned reduction of energy turnover rates was based on a psychological effect, the unrest and high locomotor activity of honeybees isolated from their nest mates. But thermodynamic backgrounds are also working: The European honeybee *Apis mellifera carnica* conquered the cooler North European parts from the Mediterranean and African area with mild winters and more stable climates. Bees had to develop strategies to store food for adverse times and to keep their colonies at temperatures above +10 °C even at ambient values deep below the freezing point. The surface-to-volume ratio of a single bee is an elegant means to avoid overheating during summer flights but becomes highly unfavourable concerning heat loss in winter. When honeybees form densely packed balls, so-called clusters, their total surface-to-volume ratio drops to 4.5% of the initial value of a single bee. Moreover, the heat conductivity in the cluster is very low and similar to that of feathers or fur due to the interlaced individual insects and to the fur-like cover of their thorax. Thus, the

bees keep a constant temperature of about 35 °C in the centre and not less than 10 °C in the mantle. Such a temperature distribution in the cluster is accurately described by a thermodynamic model consisting of the well-known cooling laws and a temperature dependent delocalised heat source [38].

Another, highly elaborated type of clustering is used by the small Japanese honeybee *Apis cerana* (about 1.2 cm and 70 mg) that is often caught as prey by the huge Japanese hornet *Vespa mandarinia* (about 5.7 cm and 1700 mg) [39–42]. If such a hornet approaches a beehive a guard bee attacks it (of course without success), but other hive bees are alarmed and rush to the predator forming a dense ball of up to 400 bees around it. This ball has a bee mass of about 28 g, a diameter of 6.4 cm and a volume of 140 cm^3. As the bees keep their metabolic rate from the hive interior (32 °C) of about 2.7 W for such a ball, and as its heat loss is low (see above), the temperature inside the ball increases over 42 °C (and a heat production of 5.4 W due to the Q_{10}-rule) to a final value of about 46 °C at 7.6 W. This heating up takes about 4 min and lasts for 20 min after which the ball starts to disintegrate leaving a dead hornet. The trick of the honeybees is that they can tolerate elevated temperatures up to 48 °C and 50 °C while the hornet endures only 45–47 °C; it dies by overheating [39–42].

The total energy consumed by the bees in their thermal war [42] is roughly 9 kJ that correspond to 0.6 g of honey (16 kJ g^{-1}) which can be collected by the 400 bees in about 40 s [43], a highly effective and ecologically clean fight against an aggressor. The European honeybee *A. mellifera carnica*, imported to Japan recently, knows about the balling trick of its relatives. But the centre of its ball reaches only 43 °C, not high enough to kill the hornet so that it has to be killed inside the cluster by two or three poisonous stings [39,40].

7. The bound dissipation function ψ_u

Since the beginning of the last century it was a common opinion that indirect calorimetry, the respiration of an organism, can be used to determine its heat production rate in an easier way than with direct calorimetry [44]. But it became obvious that there are quite often discrepancies between the direct and indirect results when they were gathered simultaneously. Theoretical considerations provoked a closer look at these discrepancies and the phase of their appearance during the experiments.

The entropy production d_iS/dt in an open system can be written as $d_iS/dt = 1/T \sum A_\rho v_\rho$ when only chemical reactions occur. T is the absolute temperature, A_ρ and v_ρ the affinity and the rate of reaction, ρ ranging from 1 to the number n of reactions in the system. Multiplying d_iS/dt with (T/V) transforms it into a volume (or mass) specific energy term and thus to the dissipation function

$$\psi = \frac{T}{V}\frac{d_iS}{dt} = \frac{1}{V}\sum A_\rho v_\rho \quad (1)$$

As A is coupled to the free enthalpy G and thus to the enthalpy H and entropy S, one arrives at a splitting of Eq. (1) into two terms

$$\psi = \psi_d + \psi_u \quad (2)$$

where ψ_d is the external dissipation function and connected to the enthalpy change and thus to that heat production rate which can be measured calorimetrically. ψ_u, connected with the entropy term in A, is the bound dissipation function (psiu-function). The subdivision in (2) makes clear that only part of the dissipation energy leaves the system during irreversible processes while a second part remains within it. For more intensive and background discussion see [44] and specially [45]. Here, just a few results shall be presented to underline the above statements and give further examples in addition to the microbial growth processes shown in Section 3.

Barott [46] and Romijn and Lokhorst [47] followed the development of hen embryos in their eggs by simultaneous direct and indirect (respiration) calorimetry. The results in Fig. 9 show the discrepancy between respiration and heat production and the calculated psiu-function. As expected from the theory of irreversible thermodynamics and the approach towards a final thermal equilibrium, the psiu-function decreases during the growth and attains a minimum level. A similar picture (Fig. 10) is obtained during the development of the German cockroach *Blattella germanica* monitored by means of a Warburg manometer and a calorimeter on the same individual insects [48]. Again, a permanent discrepancy and thus a significant psiu-function exists that becomes rather small in the

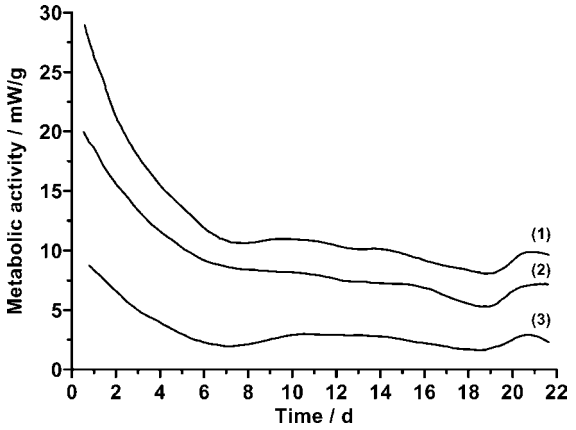

Fig. 9. Respiration (1), heat production (2), and psiu-function (3) as function of time for the development of hen embryos in their eggs (adapted from [46,47]).

life of the cockroach. The strong fluctuations in the three slopes are due to morphological changes such as repeated moultings in the course of development.

Zotin collected a large corpus of such discrepancies and stimulated the idea of a future psiumetry [44,45], involving simultaneous direct and indirect calorimetry. Although the meaning of the psiu-function is not really clear yet (see, e.g. [49]) one can discuss several lines of its origin. Energy retained in the organism may be used to accelerate biochemical reactions

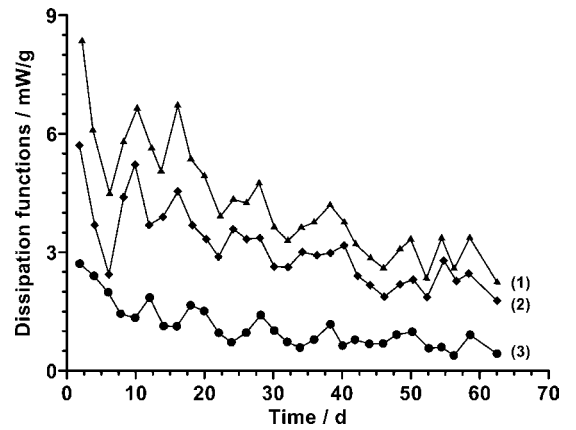

Fig. 10. Time course of the three dissipation functions given in Eq. (2) for the growth of males of the German cockroach *B. germanica* (adapted from [48]). (1) Total dissipation function; (2) external dissipation function; (3) bound dissipation (psiu-)function.

(the psiu-function being proportional to their rates). Moreover, the psiu-function depends directly on the entropy change with the degree of reaction completeness, which itself is connected with macromolecular synthesis, conformational changes of proteins and membranes or with other processes. One may conclude from the physical meaning of the psiu-function and its different dependencies that the organism with the largest psiu-function is the fittest in reproduction and growth and thus best adapted to its surrounding. High values may be correlated with health and the deviations from them with disease or weakness so that psiu-function changes may serve as a kind of diagnosis in medicine or for supervision of sportsmen. As shown earlier in this paper, the psiu-function mirrors the state of a growing microbial population, interesting in large scale industrial applications as in breweries, or during the production of cheese, single cell proteins or antibiotics [44].

8. Conclusion

It was pointed out in the Section 1 that the selection of examples from the different complexity levels of the biological systems is very personal and generated by the interest of the former Institute for Biophysics. The choice could be completely different in each section and the emphasis put on other aspects. Due to the combination of calorimetry and thermodynamics, all results originate from the classical quantitative approach and none from the more modern branch of “analytical” calorimetry where it is not the question of How much that is important, but the answers to If and to When in the time course of consecutive reactions. Typical data from this analytical field are the fingerprint-like power–time curves of growing microbial cultures, which can be used for differentiation and sometimes even for identification of microbial strains [50]. Other data are the effects on living systems in response to a specific drug or xenobiotic by a reduction (or increase) of their metabolic rates [51].

Although there are plenty of types of oscillating reactions described in the literature [13], biologically oriented calorimetry concentrated on the glycolytic oscillations mentioned above. Further investigations would be conceivable on rhythmical growth of microorganisms like the well-known clock fungi (e.g.

Sclerotinia fructigena) or of the slime mould *Dictyostelium discoideum*, and on more complex systems like predator–prey interactions, e.g. *Didinium nasutum* and *Paramecium caudatum* [52]. Interesting aspects become obvious in social insects like wasps or hornets when the energy consumption for constructing a paper-like nest cover is compared with the insulating and energy saving effects gained for the thermoregulation of the nest centre with the brood [53]. As is expected, highly developed structures with multi-layered walls, downwards pointing air pockets, nest entrances at or near to the lowest point of the envelope or use of insulating void sections in the upper part of the nest are applied [53]. Energy balances are also encountered in the development of some beetles or other insects, where the larval state has to collect and store all energy for the completely encapsulated pupal state and sometimes also for the mouthless adult animal (e.g. the great wax moth *Galleria mellonella* [54].) Further energetical aspects are touched when the influence of xenobiotic chemicals, e.g. environmental poisons like PCP (pentachlorophenol) or DDT, is monitored calorimetrically for all trophic levels of an ecological system [55].

Finally, theoretical investigations on systems far off thermal equilibrium yield predictions that have to be proved by indirect or direct calorimetry if they are not already found in the relevant literature. Zotin published a considerable number of papers and books focussed on such questions ([45], see also [56]) including changes of the (bound) dissipation function during ontogenesis, evolution, morphogenesis, insect metamorphosis, pathologic processes, diseases, regeneration, or wound healing, but also on general transition processes, pattern formation, criteria of orderliness and organization, the principle of minimum energy dissipation or questions of energy metabolism and taxonomy. This not at all exhaustive list shows that a broad field for further investigations was thus opened by Prof. Zotin—to whom this paper is dedicated—and waits to be ploughed by younger scientists after his untimely death.

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