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Metabolism and gas exchange patterns in *Rhodnius prolixus*

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ABSTRACT

Insect's metabolic rate and patterns of gas-exchange varies according to different factors such as: species, activity, mass, and temperature among others. One particular striking pattern of gas-exchange in insects is discontinuous gas-exchange cycles, for which many different hypotheses regarding their evolution have been stated. This article does not pretend to be an extensive review on the subject, rather to focus on the work performed on the haematophagous bug *Rhodnius prolixus*, a model organism used from the mid XX century until present days, with the great influence of Wigglesworth and his students/collaborator's work. I have no doubt that the renovated field of insect gas-exchange has a bright future and will advance at large gaits thank to the help of this model organism, *R. prolixus*, whose entire genome has recently being unraveled.

1. Metabolic rate: modulation and measurements

The metabolic rate of an animal is the rate at which the metabolic energy is consumed under certain circumstances, namely the "cost of living". In other words the metabolic rate is given by the sum of all biochemical reactions that occur inside the animal. Among other things, the metabolic rate of an animal is modified by the activity, the mass of the animal, the temperature, whether or not it is digesting food, the time of day, etc. (Randall et al., 2002).

The metabolic rate can be measured by direct calorimetry, *i.e.*, measuring the total heat produced by an animal in units of calories min^{-1} or kilojoules min^{-1}, or by indirect calorimetry. One technique of indirect calorimetry is to measure the gas exchange between the animal and the environment by respirometry. For example the consumption of O_2, in units such as $\mu l\ O_2\ min^{-1}$ or the production of CO_2, expressed for example in $\mu l\ CO_2\ min^{-1}$ or both. Another way to measure the CO_2 produced is by the injection of doubly radiolabeled water ($^3H_2{}^{18}O$) in animals and subsequently collecting blood samples at different time intervals. The difference in the rates of oxygen and hydrogen lost are due to CO_2 production since O_2 is lost from the animal as CO_2 and H_2O, and H_2 is lost as H_2O only. For a complete summary of the technique and it's applications see the standard reference work of Speakman (1997) for doubly radiolabeled water technique or Lighton (2008) for gas exchange measurements.

The doubly labeled water method is particularly useful for measuring average metabolic rate (it is called "field metabolic rate") over relatively long periods of time (a few days or weeks), in subjects for which other types of direct or indirect calorimetric measurements of metabolic rate would be more difficult or impossible. It has the advantage of estimating the energetic cost of living under natural conditions. On the contrary, it has the disadvantage that it is not useful for individual insects. In addition, you need to recapture the animal in healthy conditions after weeks in the wild, therefore it is an average measurement and it does not allow calculation of the energetic cost of particular activities, *e.g.*, walking, carrying a load, flying, etc.

Most insects have aerobic metabolism, even under high energetic expenditures activities such as flight, thus measuring O_2 consumption or CO_2 production, or both is a good indirect method for calculating the metabolic rate. In addition, in part due to their small size, insects have low metabolic rates and consequently small volumes of gas exchange, thus measuring CO_2 production in real time is easier and more feasible than O_2 consumption. This is because: 1) CO_2 analyzers are at least an order of magnitude more sensitive than O_2 analyzers and 2) it is possible to scrub all CO_2 from incurrent air and measure tiny concentrations above a *ca.* zero background, but it is impossible to scrub all O_2 from incurrent air and obtain meaningful aerobic metabolic information. Therefore, it is impossible to measure small concentrations (few ppm) of O_2 from a huge background under normoxic condition, *i.e.*, *ca.* 210,000 ppm of O_2, due to the low signal to noise ratio.

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However, to convert O_2 consumption or in particular CO_2 production to power (*e.g.*, μWatt) requires the knowledge of respiratory quotient (RQ). RQ can be taken from literature or measured, for example using the stop-flow technique, which is a useful technique for measuring, among other things, O_2 consumption in small insects. Briefly, a chamber is flushed with ambient air or air free of CO_2 and water, the insect is placed inside the chamber and after a time (minutes or hours, depending on the chamber's and insect's size, temperature, etc.) air from the chamber is passed through the analyzers and the O_2 consumption or CO_2 production rates are calculated based on the concentrations and time period that the insect spends on the chamber (Fig. 1). While it is not necessary for RQ measurements, to actually measure gas exchange under these conditions, the flow rate through the analyzers must be known. This allows the y axis in Fig. 1 to be in units such as ml of gas per minutes (multiplying flow rate through the analyzer, *e.g.*, in ml min^{-1} * the CO_2 concentration expressed as a fraction). If the x axis is in minutes, integrating the peak gives gas exchange in ml. Dividing by the time of chamber closure gives gas exchange rate, *e.g.* ml/time. For advantages and disadvantages of different techniques see Lighton (2008) and Lighton and Hasley (2011).

For the reasons given above, most metabolic studies, particularly those done with such small animals as individual *Drosophila* (mass *ca.* 1 mg) are conducted by the stop-flow or constant volume techniques (exception *e.g.*, Lighton, 2007; Lighton and Schilman, 2007; Schilman et al., 2011). This technique has poor temporal resolution because integrates averages of catabolic flux rates over periods of an hour or more, during which bursts of activity may lead to serious measurement overestimates (Lighton et al., 2001 and references therein). In contrast, flow-through respirometry, although far more demanding of instrumentation stability and resolution, serves to minimize these errors (Lighton, 2008), and combined with activity monitoring allows the assessment of the standard metabolic rate (SMR) and the relation between MR and activity as well dynamics changes on the pattern of gas exchange (Fig. 2).

In order to have an "instantaneous", or at least a measurement with a good temporal resolution using open-flow respirometry, it is important to take into account the lag time of the system and the time-constant of the respirometry chamber. Lag time is the time required for changes in gas concentrations inside the respirometry chamber to reach the analyzer. It is a function of the air flow rate as well as the tubing volume between the chamber and the analyzer. It is best calculated empirically by introducing a brief and sharp peak of gas (*e.g.*, CO_2) in the respirometry chamber and then measure the time for the change to appear on the analyzer, *i.e.*, CO_2 analyzer. On the other hand, the time-constant of the respirometry chamber is the time required for a step change in rate of respiratory gas exchange to reach 63% of its final value within the chamber. It is calculated by the ratio between the volume of the chamber and air flow rate (*e.g.*, the time constant of a chamber with a volume of 400 ml, through which air flows at 200 ml min^{-1}, is 2 min). Time constants of 1 or less are adequate for a proper temporal resolution of the signal. In addition, the mixing characteristics of a gas stream within a chamber can be mathematically corrected. This wash-out phenomena correction, response correction or instantaneous correction can be done by the original Z-transform method (ZT) (Bartholomew et al., 1981) or by two new methods, which are based on modifications of the original ZT method: the extension of the ZT method (EZT), and the generalized ZT method (GZT) (Pendar and Socha, 2015).

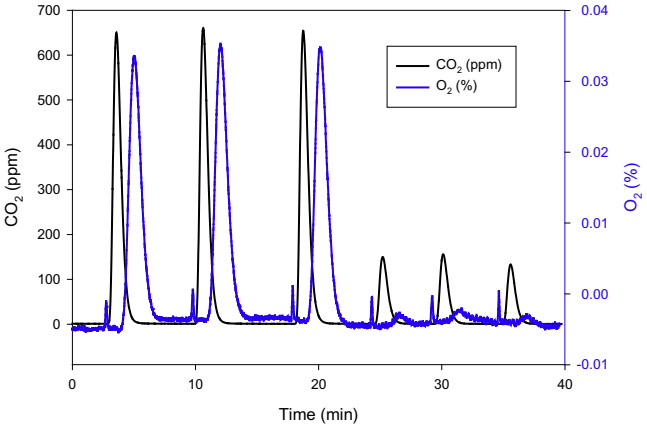

Fig. 1. CO_2 production (black) and O_2 consumption (blue) from *Drosophila melanogaster* measured by stop-flow technique. Three last peaks are controls (injected air from empty syringes), which values should be subtracted from the measurements (three first peaks; unpublished results). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

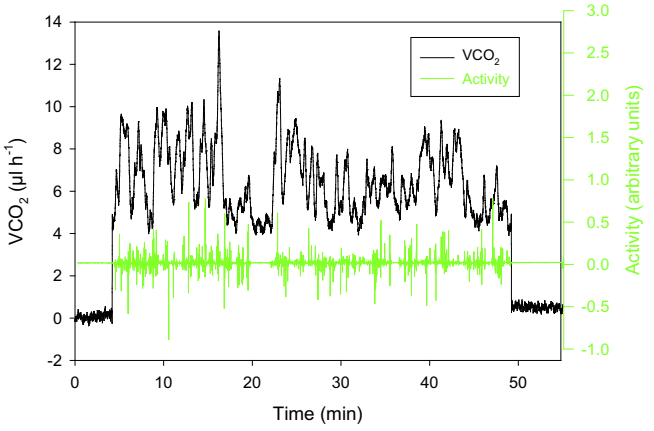

Fig. 2. Real-time recording of CO_2 production (μl h^{-1}) and activity (arbitrary units) as a function of time (min) from an individual fly, *Drosophila melanogaster* (mass = *ca.* 1 mg) at 25 °C. Note that the activity, dropping close to zero (no fluctuations) at about the 20 min mark, is accompanied by a corresponding drop in VCO_2 and SMR can be calculated (unpublished results).

2. Patterns of gas exchange in insects

In small animals, such as insects, the tracheal respiratory system allows rapid gas exchange between the cells and the atmosphere. Such rapid gaseous diffusion together with active convective ventilation (Socha et al., 2010; Harrison et al., 2013) allows insects to achieve high rates of gas exchange. For example, when leafcutter ants are cutting leaves, gas exchange increases 31-fold compared to rest (Roces and Lighton, 1995) or 20–100 times when insects are flying (Casey, 1989; Casey and Ellington, 1989; Ellington et al., 1990). The latter not only includes a higher O_2 demand of flight muscle, but also an increase for large changes in thorax temperature during flight (Harrison and Fewell, 2002).

While respiration is continuous in cells, in some insects under certain situations (for example at rest) measured as a whole organism, the gas exchange pattern is discontinuous. The intervals between bursts of gas exchange can be of only few seconds to several hours, depending on the insect (species, adult or larvae, temperature, etc.).

Also, insects are able to survive in extreme conditions of high temperature and low humidity, such as in most deserts of the earth. This feature results from the properties of the cuticle, composed of hydrocarbons and waxes that make it less permeable to water and reducing desiccation. This also contributes to the imper-

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