AUTOMATED MEASUREMENT OF OXYGEN CONSUMPTION BY THE YELLOW FEVER MOSQUITO, Aedes aegypti

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Abstract. Oxygen consumption of single mosquitoes was measured using a differential pressure transducer (DPT) connected to two small chambers. A mosquito was placed in the experimental chamber (P1) and was separated from NaOH by 4 cm² of marquisette mesh. The reference chamber (P2) contained the same amount of NaOH and the marquisette mesh but without a mosquito. When these two chambers were sealed, removed O₂ from P1 resulted in a pressure decrease with respect to P2. This pressure differential was transduced into an output voltage that was directly proportional to the amount of O₂ consumed by the mosquito. An array of eight DPTs was interfaced with an IBM 486 PC using an ADAC 5500MF analog-to-digital converter and software from ADAC (Direct View) to automate the recording procedure. We determined that our apparatus was sensitive enough to detect subtle differences in O₂ consumption in mosquitoes subjected to different physiologic conditions.

Manometric methods have been used extensively in the study of respiration and have produced a great variety of apparatus to carry out these measurements. Among the many different respirometers that have been produced, the Warburg respirometer has been the most widely used. In the early 1960s, Gilson constructed a simpler differential respirometer with a single reference flask, for multiple active flasks, and a digital readout volumeter to circumvent the tedious calculation and calibration steps. The use of manometric methods, however, has decreased because of their complexity, fragility, and cumbersome nature, and were replaced by the more sensitive, accurate, simple, and rapid spectrometric methods. Spectrometric methods, although well suited to the study of chemical and biological reactions at the cellular level, are not well suited for studies of whole organisms.

In an attempt to understand why energy costs do not balance in insects, Wightman traced the inaccuracy in the R term (respiration). He found that R was always lower when measured with a respirometer than the value obtained from consumption minus production and egesta. This suggested that all of the parameters of an energy budget, respiration was the most difficult to obtain because of the numerous factors affecting it in unpredictable ways. This was even more true for social insects. Although respiration of ant colonies did not suggest any circadian rhythm, it was possible, to observe an annual respiratory pattern characterized by a lower oxygen consumption during the cold seasons. Termites had a more complex respiratory system in which both the organism and its bacterial endosymbionts had to be considered. In the latter system, measurements of oxygen consumption have been used to study the possible roles of glucose, xylose, pyruvate, alanine, α-ketoglutarate, and acetate as energy sources for both the termite and its endosymbiotic bacterium. Metabolic studies of non-social insects, such as the house cricket, showed a strong correlation between mating and the total amount of oxygen consumed by the female. Drosophila melanogaster also has been amenable to metabolic studies. When measured following a period of anoxia, oxygen consumption in these flies decreased to 20% of the baseline control levels and returned to 60% of the baseline levels upon re-oxygenation.

Metabolic studies of adult mosquitoes have been limited, partly because of inherent difficulties including their small size, fragile nature, and the tedious process of data recording. It has been much easier to conduct such studies with larvae using manometric methods. However, larvae do not play roles in the transmission of debilitating viral, protozoan, and metazoan diseases to humans and animals. These disease agents interact with adult mosquito hosts in complex ways and warrant a multidisciplinary approach in studies of these interactions.

The cost due to parasitic infection of a vector host, a controversial issue, is often perceived rather than empirically determined because of a lack of sensitive measurement tools. This question is often answered indirectly by assessing fecundity of the parasitized vector with respect to an uninfected control, by evaluating the effects of the parasites on the longevity of the vector host, or by studying tissue damage or flight impairment of the mosquito. These studies are done, however, with large and robust parasites such as filarial nematodes and it is often perceived that smaller parasites such as arboviruses and protozoans are fairly benign to their vector host despite their uptake of nutrients and multiplication within their hosts. The development of more sensitive tools for measuring metabolic burden would enable one to study these less overt effects and could provide insight towards further investigations into biochemical and molecular mechanisms influencing vector-parasite interactions.

Consequently, we were interested in developing a cheap, rapid, sensitive, and automated method for measuring total metabolic activity in individual adult mosquitoes. A holistic physiologic process such as total oxygen consumption can be dissected, at least in principle, into smaller contributing components as long as the measurement device possesses the required sensitivity. Although the total amount of oxygen consumed by a single mosquito is very small, herein we demonstrate that our apparatus is capable of detecting differences in oxygen consumed by mosquitoes undergoing subtle physiologic changes.

MATERIALS AND METHODS

Mosquito stocks. The Liverpool strain of Aedes aegypti was reared at 26.5 ± 0.5°C and 80 ± 5% relative humidity.
in a walk-in environmental chamber with a 16-hr light and 8-hr dark light cycle. Males and females were separated during the pupal stage (day 6 post-hatching) and were maintained on a 0.3 M sucrose diet post-emergence. Mosquitoes were fed on anesthetized gerbils for the blood-fed group.

**Apparatus.** The apparatus consisted of an array of eight Honeywell (Freeport, IL) differential pressure transducers, type 163PC01D36, with a pressure range of $-12.55$ to $+12.55$ cm of water, and biased by a regulated 8 volt supply. Signal output, which was a voltage proportional to pressure differential, was fed to an 8-channel analog to digital converter with 12 bit resolution (ADAC model 5500MF; American Data Acquisition Corporation, Woburn, MA) running Direct View (American Data Acquisition Corporation) software on a 486 PC. Identical 0.6 ml cylindrical chambers (lower tip of Labcraft® microliter pipet aspirator; Drummond Scientific Co., Broomall, PA) were fitted to the two transducer ports at one end, with removable stoppers at the other end. These constituted the experimental and reference chambers (Figures 1 and 2). Each stopper was penetrated by a capillary tube that could be sealed to isolate the chambers from the atmosphere for calibration or an experimental run.

**Measurement of oxygen consumption.** The chambers were setup with 0.35 g of solid NaOH and 4 cm$^2$ of marquisette mesh to separate the mosquito from the corrosive chemical. Before the introduction of mosquitoes, the chambers were sealed and the NaOH removed all the CO$_2$. Subsequently, the output voltage remained constant throughout time (Figure 3). Leaks were easily detected after this run. Four mosquitoes of each of the groups were aspirated into clear vials and ice anesthetized. Inactive mosquitoes were carefully transferred into the experimental chambers and sealed. Data were collected over a 45-min period. The first 15 min were disregarded because this was a period of equilibrium of the NaOH and acclimation by the mosquitoes. The range from minutes 16 to 45 was multiplied by 2 to determine the hourly output voltage change. This allowed us to avoid having an O$_2$ concentration in the chambers decrease below a critical level. The data from some chambers, characterized by multiple-modalities of the curves, indicated O$_2$ stress and were not included in the analyses.

**Calibration and formulas.** To convert the output voltage change into microliters of O$_2$ per hour, we calibrated each transducer with a manometer to plot the pressure differential in centimeters of water with respect to the O$_2$ consumed. Because the graph is highly linear, regression analysis yielded an $r^2$ value of 0.999 (Figure 4) and a concise formula to obtain pressure differential from any output voltage.
The number of oxygen molecules that the mosquito consumes is given by \( N = 4.58 \times 10^{19} \times V \times P/T \) where \( V \) is the volume occupied by air in the chamber, \( P \) is the pressure differential in centimeters of water, and \( T \) is the temperature of the milieu in °K.14 The number of microliters of \( O_2 \) consumed per hour is calculated knowing that a mole of any gas contains an Avogadro number of molecules and occupies a volume of 22.4 L at standard temperature and pressure.

**Statistical analyses.** SigmaStat® (SPSS, Inc., Chicago, IL) was used to perform the statistical analyses. A one-way analysis of variance (ANOVA) was performed on the data obtained from the output voltages when the empty chambers were sealed. The calibration data were analyzed using linear regression. Repeated measurement analysis of variance (RM-ANOVA) was performed on the blood-fed versus sucrose-fed and the males and females data. Finally, we used the unpaired \( t \)-test to compare mean oxygen consumption based on body weights of males and females.

**Figure 2.** Aluminum box with eight pressure sensors and a plexiglass top. The power supply box rests on the hard-drive of the IBM PC 486 (left). The digital multi-meter at the back is used to test the output voltages of the transducers.

**Figure 3.** Output voltage when the chambers are sealed, without a mosquito, over a 45-min period.

**Figure 4.** Calibration of a pressure sensor when the output voltage is plotted with respect to change in pressure in centimeters of water. Linear regression yields a \( r^2 \) value of 0.99 with the conversion formula: centimeters of water = 2.54 \( \times \) \(-7.36 + (5.36 \times [\text{output voltage} + 3.47])\).
**RESULTS**

**Transducer stability.** Before measuring O₂ consumption of mosquitoes, we calibrated each of the eight transducers and a one-way ANOVA verified that they were not statistically different from one another ($P = 0.621$). For more accurate values of the pressure differential, we used nonetheless the regression formula of each transducer. When the chambers without mosquitoes were sealed, the output voltage stabilized well before 15 min and stayed that way for more than 2 hr (Figure 3). It was noted, however, that the output voltage drifted slightly when the plexiglass top was opened, thereby exposing the transducers and the chambers to more fluctuation of atmospheric air in the laboratory.

**Response of *Ae. aegypti* to anoxic conditions.** When the O₂ level in the chamber decreased to a critical point, the mosquitoes reacted by lowering their respiratory rate and the slope of the curve representing oxygen consumption with respect to time decreased significantly. Periodically, the slope increased to levels similar to the oxygen-rich state and as time progressed, the curve deviated from the linear pattern and resumed a more erratic behavior marked by multiple modes.

**Oxygen consumption of blood-fed and sucrose-fed mosquitoes.** Blood-fed mosquitoes consumed significantly greater amounts of oxygen compared with sucrose-fed mosquitoes (Figure 5). Moreover, oxygen consumption of both sucrose-fed and blood-fed *Ae. aegypti* decreased gradually with the number of days elapsed, suggesting that the rate of oxygen consumption was dependent on aging. Because both groups were fed on sucrose after the initial blood meal, differences are due to the major metabolic burden associated with egg development in blood-fed mosquitoes.

**Oxygen consumption of sucrose-fed male and female mosquitoes.** Female mosquitoes consumed significantly greater amounts of oxygen ($P < 0.0001$) (Figure 6). Because females *Ae. aegypti* are significantly larger than males, the difference in rates of oxygen consumed might be attributed, in part, to differences in body size. When the rate of oxygen consumed per individual mosquitoes was calibrated with body weight, statistical analysis of the data revealed no differences (Table 1) in oxygen consumption between males and females.

**DISCUSSION**

Although oxygen consumption of several insects has been studied using a variety of techniques, the uniqueness of our work stems from the automation, relatively low cost (< $1,200), sensitivity, ability to evaluate the same individual throughout time, and ease with which experiments can be conducted. The results of this work show that the respirometer is sensitive, stable, and exhibits linearity between the output voltage and pressure differential. Although it was known that the design of some differential pressure transducers can cause measurement errors under certain circumstances, such as intermittent positive pressure ventilation,¹⁵ use of the small, equal-sized chambers allowed us to circumvent this problem.

The apparatus that we describe herein will allow us to conduct detailed experiments to test the cost (if any) associated with parasite development in adult mosquitoes when

**Table 1**

Comparison of the rates of oxygen consumption as a function of body weight between male and female *Aedes aegypti*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Male mean ± SD (n = 20)</th>
<th>Female mean ± SD (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (mg)</td>
<td>1.29 ± 0.135</td>
<td>2.61 ± 0.317</td>
</tr>
<tr>
<td>O₂ consumed (µl/hr/mg)</td>
<td>0.65 ± 0.0508</td>
<td>0.68 ± 0.0574</td>
</tr>
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* The difference in the mean values of O₂ consumption between the two groups is not statistically significant ($P = 0.1477$, by unpaired t-test).
interacting with parasites or when subjected to stress-inducing biological events. Van Handel studied metabolism of *Culex pipiens quinquefasciatus* by measuring the amount of lipids and glycogen consumed by the developing embryo. He concluded that embryonic respiration was supported largely by lipids (90%) and glycogen (10%). Thus, during their development in a nutrient deficient environment, the resistance of mosquito larvae to starvation depended strongly on stored energy, mainly lipids, and on decreasing their respiratory rate.

More studies have been conducted on the respiration of *Drosophila*. Respiration of larval salivary glands of *Drosophila* was correlated with the activity of specific genome loci. When measured after the addition of isocitrate (an intermediate of the citric acid cycle) and tyrosine to the incubation medium, oxygen consumption of *Drosophila* salivary glands, previously subjected to anaerobiosis in vivo, was much greater compared with oxygen consumption of the salivary glands of the control larvae. Inhibition of proteins and RNA suppressed the stimulatory effects of isocitrate and tyrosine, suggesting that some of the puffs in the polytene chromosomes reflected gene activity for the use of isocitrate and tyrosine for respiration during conditions of stress. The sensitivity of our apparatus suggests that we might be able to assess O2 consumption in salivary glands of individual mosquitoes uninfected or infected with *Plasmodium* sporozoites.

The role of oxidative stress in the induction of heat-shock proteins in *Drosophila Kc* cells was evaluated by comparing the effects of temperature stress and reoxygenation following a period of anoxia, on cellular respiration, thiols status, and accumulation of heat-shock proteins. Furthermore, a respirometric study of oxygen consumption on *Drosophila Kc* cells following a period of anoxia have suggested that the generation of superoxide ions during reoxygenation may ultimately lead to the synthesis of heat-shock proteins, although indirectly. Oxidative damage played an important role in regulating the longevity of *D. melanogaster*. It was shown that the lifespan of *Drosophila* was inversely correlated to its metabolic rate. Thus, the rate of oxygen consumption by adult fruit flies was directly related to oxidative damage induced by oxygen radicals and by-products of respiration, as shown by higher titers of hydrogen peroxides and lipofuscin in flies kept under conditions of higher metabolic rate. P element transformation of *D. melanogaster* with bovine cDNA for Cu/Zn superoxide dismutase, an enzyme that catalyzes the dismutation of superoxide radicals to hydrogen peroxide and water, resulted in resistance to oxidative damage and thus increase in the life span of these transgenic flies.

It must be noted, however that this system is only capable of measuring resting metabolism. Special care must be taken to restrict motility once the mosquitoes are placed into the chambers. The marquisette mesh serves this purpose in addition to separating the mosquitoes from the NaOH. The length of time of measurements is limited because the rate of O2 consumption changes when CO2 levels are low. Gas chromatography can be used to circumvent this problem, but the amount of CO2 produced by the mosquito is not as accurate a measurement of respiratory activity as O2 consumption. The preference of using O2 consumption to estimate overall metabolism over the release of CO2 results from the fact that unoxidized and incompletely oxidized carbon will not be released, resulting in an underestimation of the overall metabolic activity. Furthermore, the release of carbon gases other than CO2 in closed-vessel respirometric systems equipped with an absorbent for CO2 released will result in an underestimation of carbon metabolism directly, and indirectly in an underestimation of oxygen consumption. The production of CO2 (output) depends on more variables such as anaerobism, cataabolism, and the water content of the mosquito. Depending on the purpose of studies, our apparatus can be easily modified to measure CO2 levels produced by mosquitoes.

Acknowledgments: We thank Dr. Carl Lowenberger for assistance with the statistics, and Linda Christensen, Jody Chiles, and Andrew Taft for technical assistance.

Financial support: This study was supported in part by National Institutes of Health grants AI-19769 and AI-28781.

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