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FURTHER INVESTIGATION ON THE RESPIRATION IN PUPAE OF GALLERIA MELLONELLA: RECORDINGS OF BODY LENGTH CHANGES, SPIRACULAR RHYTHMS AND CO₂ RELEASE

Simultaneous records of pupal length changes and gaseous flow through each spiracle selectively were made by means of a displacement transducer and a thermocouple thermograph. At the moment of the sudden body lengthening an expiration stroke occurred lasting 1—3 s, followed only then by an inspiration stroke. The 7th abdominal spiracles (7AL and 7AR) often revealed only a strong expiration stroke with no inspiration, while all other spiracles showed a weak expiration and strong inspiration strokes. No difference was detected in spiracular ventilation between either body side. In the interburst period the CO_2 absolute level was insignificant as confirmed by the records ment transducer.
of the heat conductivity detector (catharometer) and infrared gas analyzer (IRGA).
The pupae exhibited regular periods of muscular contractions, independently of gas exchange cycles, but only stronger contractions functioned as a pumping ventilation and were recorded, as a frequent peaks, with an actographic respirometer and a displace-

Introduction

External gas exchange has so far been studied in detail only in a limited number on insect species and these investigations have revealed a great variety of gas exchange patterns. One of the central problems is the role of gaseous diffusion and muscular ventilation, and relations between them. It is obvious that most insects can make use of both the mentioned principal mechanisms of respiration.

To reveal the rhythms of discontinuous respiration, various specific methods are required that are readily applicable in studying large insects, especially large lepidopteran pupae (*Sphingidae, Saturniidae, Pieridae*) (Schneiderman, 1960; Levy and Schneiderman, 1966; Куузик, 1977; Miller, 1981; Kestler, 1985; Slama, 1988).

Cyclic CO₂ release during discontinuous gas exchange is commonly recorded with thermal conductivity detectors: diaferometer (Punt, 1950, 1956) or chromatographic catharometer (Куузик, 1976). Cyclic CO₂ emission has also been recorded with IRGA (Hamilton, 1959, 1960, 1964; Lighton, 1988) or differential IRGA (Kestler, 1985). The same methods were needed to detect intermittent O₂ uptake (Punt et al., 1957; Bartholomew et al., 1984; Lighton, 1987; Kestler, 1985; Slama, 1988).

Spiracular movements coordinated with ventilation have been recorded by means of a thermistor sealed into tracheae (Miller, 1974) or a thermistor placed in the orifice of a teflon tubing leading to the spiracle (Slama, 1988).

Pupal length changes due to tracheal volume changes have been recorded with the help of force-displacement transducers (Brockway and Schneiderman, 1967) or isotonic transducers (Slama, 1988) attached to the tip of the abdomen.

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Intratracheal barometric pressure has been measured directly, using manometer tubes in the pupae of *Hyalophora cecropia* by cannulating the tracheal system and using electronic transducers (Brockway and Schneiderman, 1967).

It is well known that any influence of experimental handling or "stress" can induce activity or raise the metabolic rate (Kestler, 1985), and must thus affect the rhythms of gas exchange. The smaller is the insect the more can any recording device attached to the insect influence the gas exchange. The various methods for the automatic recording of the respiration behaviour in a freely moving insect have been employed by various authors (Kestler, 1985; Lighton, 1988; Slama, 1988).

Body movements must often be controlled during respirometric studies while respiratory movements must be discriminated from other body movements. Lighton (1988) used a special actograph based on near-infrared reflection. In an earlier study (Kuusik et al., 1991) we have described an actographic respirometer to record the moving activity, breathing rhythms and also the O_2 uptake level in a freely moving insect.

In this report we describe some results obtained with electronic transducers which can monitor spiracular movements, body length changes and the rhythms of active and passive ventilatory movements in the pupae of *Galleria mellonella* with an average body mass of 0.1—0.11 g.

Material and methods

Insects. Wax moths (*Galleria mellonella* L.) were reared at 29 °C and $70 \pm 5 \%$ R. H. in constant darkness on a semiartificial diet (Sehnal, 1966). The pupae of known age (± 2 h) were used in the experiments.

Respirometry. Our methods of respirometry have, in general, been described earlier (Kuusik et al., 1991). Here we are going to add some details which are essential from the technological aspect. Since available standard photodiodes proved unsuitable for our transducers as a sensitive element a transistor (KT 302A) with light-sensitive area of only 0.26 mm² was used as a photoresistor. An aperture ($\varnothing \approx 0.5$ mm) had to be drilled in the cover of the transistor. The glass capillary near the phototransistor, glued to capillary, was covered with black lacquer, leaving a tiny hole upon the photoresistor for a narrow light beam directed through the glass capillary. An ethanol meniscus in the capillary partly shutters the light according to the principle of total inner reflection of light. In this way the smallest respiratory movements of an insect were recorded as sharp peaks. Different types of movement such as the wriggling of the abdomen or contractions of ventral abdominal intersegmental muscles ("bending" movements), or any kind of muscular contractions due to external mechanical or chemical excitation were easily distinguishable. Also, gas exchange cycles showed characteristic patterns on the recordings. Simultaneously, the average O_2 uptake level was measured by the electrolytic compensation of consumed O_2.

The length of the CO_2 peaks on the respirograms depended on the distance between the insect and the CO_2 absorbent. In order to record the respiratory movements in greater detail, the CO_2 peaks were made shorter or were omitted altogether by increasing the CO_2 absorbent surface and by shortening the distance between the insect and the absorbent.

IRGA. In the recordings of the infrared gas analyzer (Infralyt 4, Dessau, Juncolor) the baseline was calibrated only in such cases when the duration of the CO_2 burst cycle lasted longer than 15 sec, and when each CO_2 burst did not overlap its neighbouring bursts. In other cases IRGA recordings were considered only as qualitative ones.

Catharometry. As a thermal conductivity detector, a gas chromatographic catharometer (Biochrom, SU) adjusted for entomological research in laboratory was used. For this purpose the wolfram wire was replaced by a single-fold platinum ($\varnothing$ 0.06 mm) spiral (diam. of coil 1.2 mm). The resistance of the platinum spiral was 3.6 Ω, and the Wheatstone bridge was fed by the stabilized voltage of 4—5 V. The 400—450 mA current was sufficient to guarantee the recording of discontinuous CO_2 emission in single small insects such as the pupae of *Galleria mellonella*. The flow rate of the carrier air current was 25—30 ml per min. This flow rate was sufficient to record each CO_2 burst separately without overlapping and, thus, the interburst plateau period had a sufficient duration for measuring the level of the baseline of CO_2 emission during the interburst period. Ascarit was used as an absorbent of CO_2. The general scheme of the flowthrough system was designed on the basis of Punt's diaferometric device (Punt, 1950, 1956). A glass vial (internal volume 0.6 ml) was used as a respiratory chamber. During CO_2 output recordings the insect was visible under a stereoscope-microscope. The respiration chamber was connected directly to the inflow tube of the catharometer.

The catharometer together with the respiration chamber were placed in a thermostat while temperature was kept constant within the range of 15 to 30 °C. A copper thermal equilibration coil guaranteed the thermostating temperature of the gas stream directed into the respiration chamber.

Thermography for monitoring the air passage through spiracles.
Instead of a heated thermistor used for following the route of ventilating air streams in insects (Miller, 1974; Slama, 1988), we preferred a method of Const-Cu thermocouple. The welded junction of the thermocouple has a diameter of 0.3—0.35 mm, and one junction was placed upon the stigmal plate of the spiracle at a distance of 0.2—0.3 mm, while reference junction was at a distance of 20 mm from the body surface. The signal from the thermocouple was amplified by a nanovoltampermeter (N-341). A clear inspiration of expiration stroke caused a change of the thermopotential within the range of 0.02—0.05 μV, which corresponded to the $\Delta t°$ of 0.0005—0.0013 °C. The movement of the air stream between the stigmal plate and the thermocouple junction caused either positive or negative temperature changes on the thermocouple, while the insect body temperature was 0.005—0.02 °C higher than the ambient temperature. Thus, the thermocouple served as a thermograph for recording expiration and inspiration rhythms from single spiracles due to warming or cooling effects, respectively.

The insect was fixed to the glass substrate and the thermocouple device was placed in a vessel of 1—2 l volume covered with organic glass. The friction between the thermocouple and the body surface was avoided by means of special wire props besides the welding point.

Measurements of body length changes. The electronic displacement transducer for recording changes of the body length was constructed on the same principle as the actographic respirometer (Fig. 1). A metal light screen (copper folium) was glued to the top of the abdomen with wound-glue (BF — 6); likewise the top of the head was glued to the transducer block. The light directed to the photoresistor was screened off or opened by means of the mentioned light screen according to body lengthening or shortening due to respiration movements.

The calibration curve for the recorder (Fig. 2) was provided empirically by displacement measurements as the photosensitive element was gradually screened. The plotline made calculations of absolute body length changes more exact.
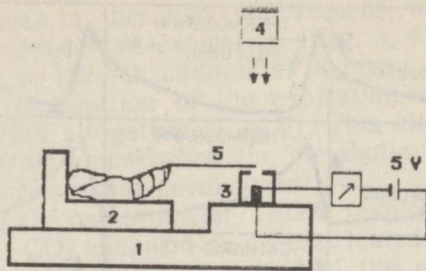
Fig. 1. The displacement transducer for recording changes of the body length. *1* — basis, *2* — transducer block, *3* — photoresistor, *4* — light source, *5* — light screen.
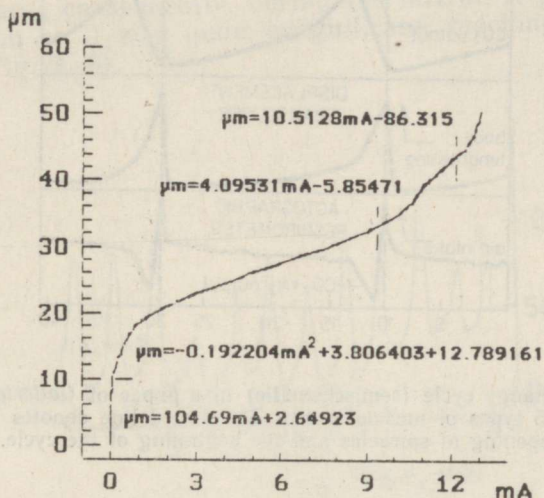

Fig. 2. Calibration curve calculated for body length and current changes in the photo-resistor circuit.

The sudden lengthening of the abdomen of a pupa due to the synchronous opening of the spiracles caused a quick downward movement of the pen driver as a result of shuttering the photoresistor. The recorder was calibrated in μm and thus the relative changes of the body length were estimated.

Simultaneously were recorded spiracular movements with body length changes as well as spiracular movements with respirometric measurements.

The sample records for photos were selected as typical from measurements on at least 12 pupae of both sexes.

Results

Simultaneous recording of the pupal length and temperature near the stigmal plate revealed a sudden upward movement of the pen driver of thermograph during ≈0.5 sec after the rapid pupal lengthening (Fig. 3). The mentioned up-peak occurred due to the warming effect of expiration. Expiration was immediately followed by a fast inspiration stroke which caused a cooling effect in the thermograph (Fig. 3, 4). Microscopic observations in catharometric measurements also revealed that the outburst of CO_2 began at the moment of the sudden body lengthening.
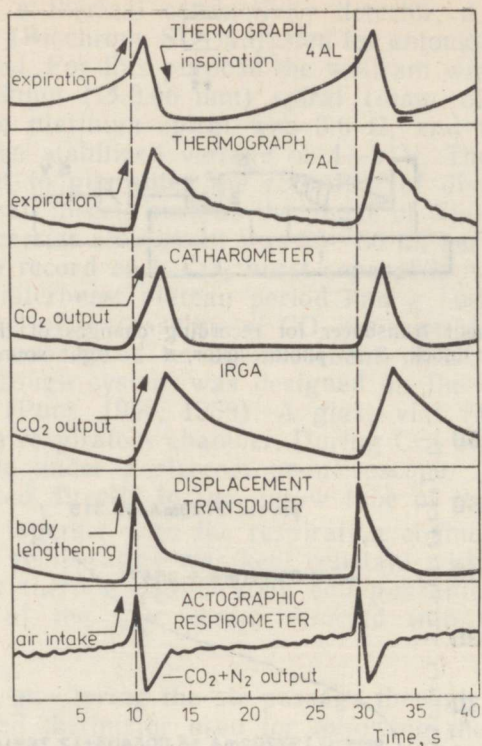
Fig. 3. The gas exchange cycle (semischematic) in a pupae of *Galleria mellonella* by the records of 5 types of measurements. The dotted line denotes the moment of the opening of spiracles and the beginning of the cycle.
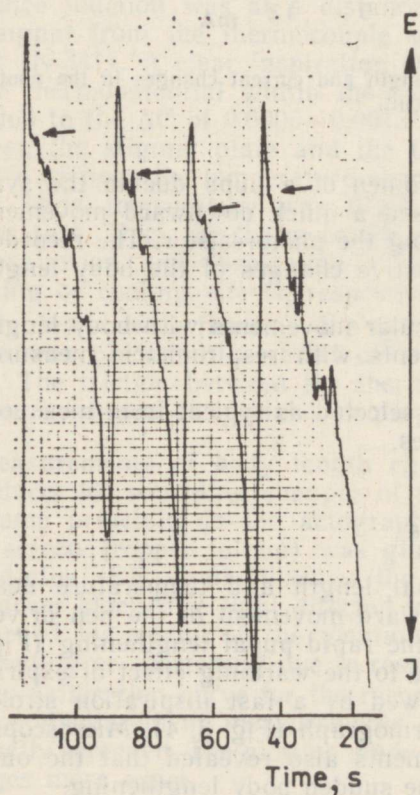

Fig. 4. The thermograph record of air passage through 4AR spiracle. *E* — expiration movement; *I* — inspiration movement. Arrows indicate the beginnings of expiration strokes.
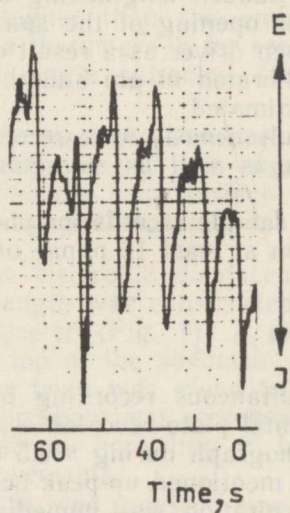

Fig. 5. Recording of dominating expiration from 7AL spiracle.

All abdominal spiracles on either body side showed a similar pattern of gas exchange seen on thermographic records, while the inspiration was always more expressed than expiration (Fig. 3, 4). By the way of exception the spiracles of the 7th abdominal segment often showed a specific pattern with the domination of the expiration stroke while inspiration was weak or omitted altogether (Fig. 5). This kind of gas exchange was typical for 30—50 hr pupae when the metabolic level was decreasing to the lower level in the pupal development.

The records of the catharometer, IRGA, respirometer and thermograph demonstrated that CO_2 emission as a burst lasted only 1—3 s at $20 \pm 2\,°C$, and it did not depend on the duration of the gas exchange cycle. The intake of air through spiracles (inspiration) lasted 2—3 s, and this occurred always after the expiration stroke.

The records of the catharometer showed that during the period of body shortening and, consequently, during the interburst period there was no CO_2 emission or it was quite insignificant, reaching scarcely over the zero-line (Fig. 3, 6).
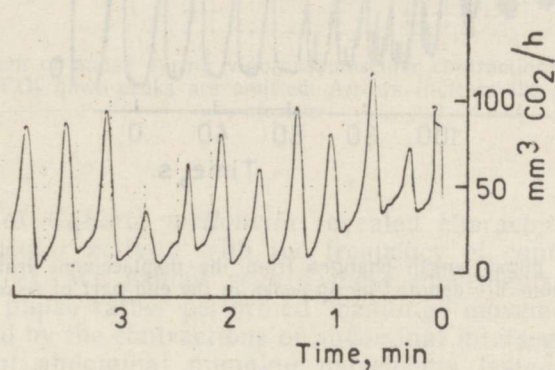
Fig. 6. Continuous catharometer recordings of CO_2 output from a male pupa of *Galleria mellonella* (body mass 85 mg; mean output 455 µl $CO_2/g/h$).
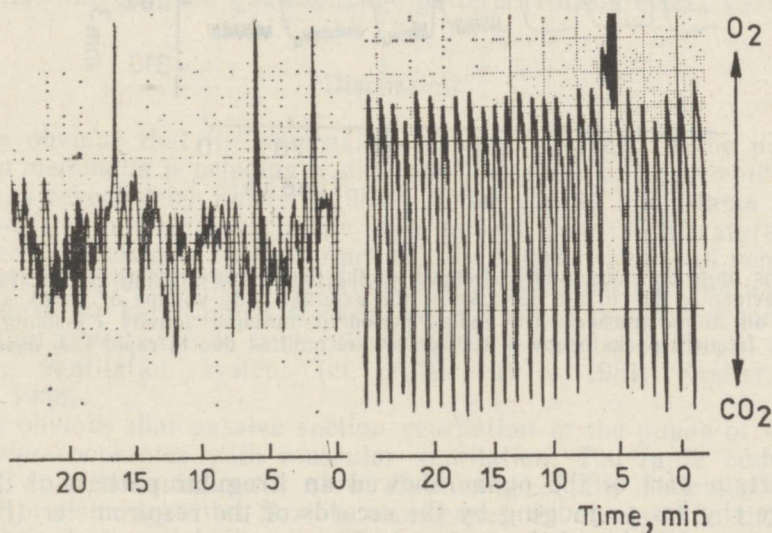
Fig. 7. The irregural gas exchange rhythm (left) and the clear gas exchange cycles (right).

The records of the actographic respirometer, thermograph and displacement transducer revealed that at the moment of the sudden body lengthening together with the CO_2 burst the pen driver of respirometer made a quick and vigorous upward movement (Fig. 3).

The body length changes in pupae averaging 16 mm occurred within the limits of 3.5 μm (i. e. 0.02% of body length). The total lengthening took place in two steps: 1) the rapid one, where the body lengthened 3 μm ($\approx 80\%$ from total lengthening), 2) the rest slow lengthening during half cycle period (Fig. 8).
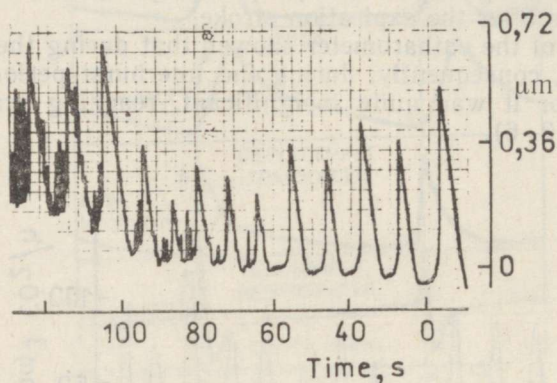

Fig. 8. Records of pupal length changes from the displacement transducer. Muscular contractions are denoted as up-peaks in the end part of recordings.
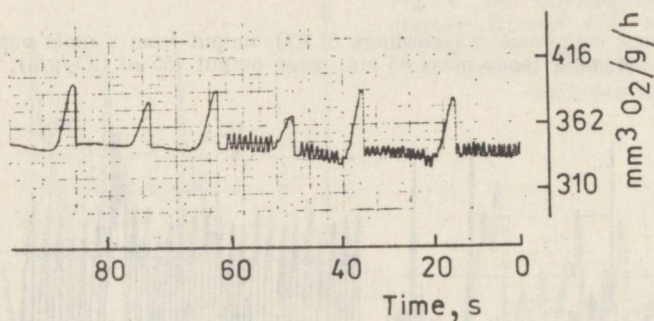

Fig. 9. The up-peaks note sudden decrease of the barometric pressure in the respiration chamber evidently due to the decrease of the external body volume or (and) a sudden intake of air in the tracheae. The ending period of muscular activity ("bending" movements) as frequent peaks is given. CO_2 peaks are omitted due to rapid CO_2 absorptsion.

A certain part of the pupae showed an irregular pattern of the gas exchange rhythm as judging by the records of the respirometer (Fig. 7), catharometer, IRGA and thermograph. There were also a number of pupae with fully arhythmic gas exchange; in this case all the above-mentioned records showed a baseline without considerable up- or down-peaks.
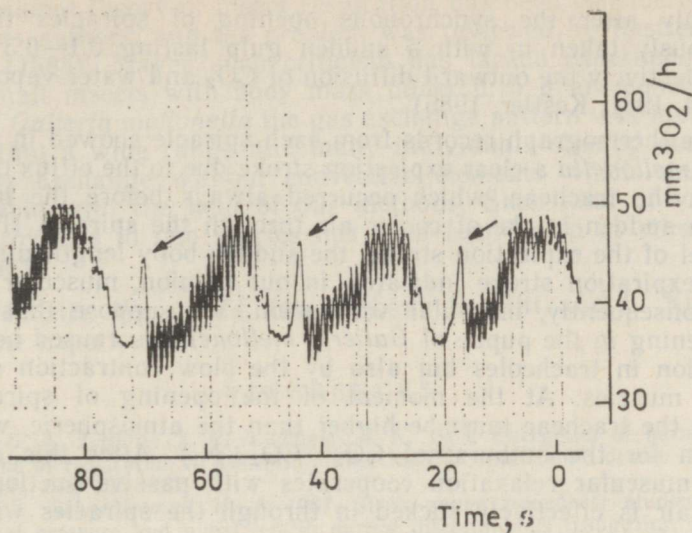
Fig. 10. Respirogram of pupae during vigorous muscular contractions (freaquent peaks) due to which the CO_2 down-peaks are omitted. Arrows indicate the peaks of air intake strokes.

The pupae of *Galleria mellonella* revealed characteristic periods of abdominal muscular activity with the frequency of contractions 30—60 per min (Fig. 9). We cannot regard these movements as telescoping ones, since the pupae rather performed "bending" movements which were evidently caused by the contractions of abdominal intersegmental muscles. Such periods of abdominal pumping movements lasted 1—5 min and were separated by periods of "stillness" lasting 3—8 min depending on the individual. When pumping movements were more vigorous, the typical CO_2 peaks shortened or disappeared altogether from respirograms (Fig. 10). Weak abdominal movements ("bending" and saw-tooth pattern) did not disturb the common gas exchange pattern (Kuusik et al., 1991).

Discussion

It is obvious that the external gas exchange cycle in the pupae of *Galleria mellonella* is principally different from those of large lepidopteran pupae, as revealed by Kestler (1985), Miller (1974) and Slama (1988), and also from other gas exchange types known from the literature.

There are known at least two principally different modes of ventilation in insects: passive suction ventilation and active muscular ventilation by dorsoventral or longitudinal strokes. Neither of the mentioned convective ventilation types exclude each other and they can be combined into an effective ventilation system (cf. Miller, 1974, 1981; Kestler, 1985; Slama, 1988).

It is obvious that passive suction ventilation in the pupae of *Galleria mellonella* cooperates with muscular ventilation. The rapid body lengthening and the subsequent slow shortening of the abdomen on the account of intersegmental flexible membranes, as observed in the pupae of *Galleria mellonella* (Fig. 8), is typical of passive suction ventilation. However, in the case of typical suction ventilation in large lepidopteran pupae partial vacuum occurs in the tracheae due to the consumption of

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oxygen; only after the synchronous opening of spiracles the air is instantaneously taken in with a sudden gulp lasting 0.1—0.3 s, which prevents effectively the outward diffusion of CO_2 and water vapor (rewiev Miller, 1974, 1981; Kestler, 1985).

Separate thermograph records from each spiracle showed in the pupae of *Galleria mellonella* a clear expiration stroke due to the efflux of warmed gases from the tracheae, which occurred always before the inspiration stroke — a sudden intake of cooler air through the spiracle (Fig. 3, 4). At the onset of the expiration stroke, the sudden body lengthening occurs. The rapid expiration stroke indicates, in our opinion, muscular contraction and, consequently, muscular ventilation. We suppose that the slow body shortening in the pupae of *Galleria mellonella* is caused not only by O_2 absorption in tracheoles but also by the slow contraction of certain abdominal muscles. At the moment of the opening of spiracles, the pressure in the tracheae must be higher than the atmospheric, which is a precondition for the outburst of CO_2 (CO_2+N_2). After this expiration movement muscular relaxation cooperates with passive suction ventilation when air is effectively sucked in through the spiracles which were opened only for a short duration.

The records of the catharometer, IRGA and actographic respirometer confirmed that the outburst of CO_2 within a gas exchange cycle lasted only 1—3 s (Fig. 6). During the interburst period an insignificant level of CO_2, obviously due to the microbursts of CO_2 (CO_2+N_2), was observed. Such microbursts of CO_2 were clearly revealed on the records of IRGA (Kuusik et al., 1991) and from highly sensitive thermographic measurements close to spiracles (Fig. 4).

Judging by the records of several methods, as shown in Fig. 3, it was most difficult to account for the sudden up-peak on the records of the actographic respirometer, which occurred exactly at the moment of the body lengthening. This upward peak was the result of a sudden decrease in the barographic pressure within the respiration chamber due to the decrease of the external body volume of the pupae but the up-peak could well be the result of the sudden air intake in the tracheae. By the other hand the decrease of body volume of pupa has to occur together with the body shortening but this contradiction remained for us unsolved.

There is evidence, that abdominal "bending" movements functioned periodically as pumping ventilation, especially during vigorous muscular contractions when CO_2 cycles disappeared.

In many insects spiracle valves move synchronously with ventilation: some open with inspiration, others with expiration (Miller, 1974), while a few species exhibit unidirectional ventilation. Large sphingid pupae often use a single "master" spiracle for prolonged discontinuous inspirations during the interburst period, while all other spiracles are tightly closed (Slama, 1988).

In the pupae of *Galleria mellonella* we could not detect essential differences in spiracular movements in different body sides. Inspiration strokes were most vigorous on the 3rd and 4th abdominal segments and weakest on the 5th and 6th segments. In the pupae whose spiracles 7AL and 7AR showed only an extensive expiration stroke without any inspiration stroke (Fig. 5) we suppose unidirectional ventilation, which occurs most commonly between 35 and 45 hrs of the pupal stage when the metabolic level is in the decreasing phase.

In the pupae with irregular pattern of gas exchange we presume slow and irregular muscular contraction and relaxation. In the pupae with fully arhythmic respiration without any detectable muscular contractions and without suction ventilation we suppose an diffusive gas exchange dominating while convective gas exchange played only a second rate role.

The diffusional theory of insect respiration has been considered doubtful by various authors, and the problem was reviewed by Kestler (1985) and Slama (1988). Maybe such criticism has lacked experimental data as far as small insects with body mass under 0.15 g are concerned. In the pupae of *Galleria mellonella* the gas exchange pattern was individually modified within a wide range and many individuals showed dominating diffusive gas exchange without convective elements which could have been detectable with the help of our methods. Such kind of variety of respiration rhythms in one species has been found also in other insects (Tartes, 1990).

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VAHALEEDIKU *(GALLERIA MELLONELLA)* NUKU HINGAMISE UURIMINE: KEHA PIKKUSE MUUTUMISE, HINGAMISAVADE TÖÖRÜTMI JA CO_2 ERALDUMISE REGISTREERIMINE

Jätkati vahaleediku nuku gaasivahetuse (GV) tsüklite uurimist, kasutades elektronnihkandurit ventilatsiooniga seotud keha pikkuse rütmilise muutumise mõõtmisel, termopaarset termograafi hingamisavade töörütmi jälgimisel ja kataromeetrit CO_2 tsüklite registreerimisel. Enne õhu sisseimemist trahheedesse toimub CO_2 väljapaiskumine (eeldatavasti) sundkonvektsiooni teel. Tagakeha seitsmenda lüli hingamisavadel registreeriti GV tsüklis tugev välja- ja nõrk sissehingamine, ülejäänud lülide hingamisavadest aga nõrk välja- ja tugev sissehingamine. Atsüklilise ja pideva GV-ga isendeil eeldatakse puhta difusiooni, tsüklilise GV-ga isendeil aga sundkonvektsiooni domineerimist.

Ааре КУУЗИК, Лууле МЕТСПАЛУ, Кюлли ХИЙЕСААР, Урмас ТАРТЕС

ДАЛЬНЕЙШИЕ ИССЛЕДОВАНИЯ ДЫХАНИЯ КУКОЛОК ПЧЕЛИНОЙ ОГНЕВКИ *GALLERIA MELLONELLA L.*: РЕГИСТРАЦИЯ ИЗМЕНЕНИЙ ДЛИНЫ ТЕЛА, РИТМА РАБОТЫ ДЫХАЛЕЦ И ВЫДЕЛЕНИЯ CO_2

У куколок пчелиной огневки (ПО) внешний газообмен (ВГ) существенно отличается от ВГ целого ряда крупных видов чешуекрылых (*Pieridae, Sphingidae, Saturnidae*).

Применением датчика смещения и термопарного термографа получена синхронная запись ритмического изменения длины тела и движения газов через дыхальцы куколки ПО. Установлено, что в момент быстрого удлинения тела (на 3 мкм) в результате синхронного открывания дыхалец начинается выделение CO_2, продолжающееся 1—3 с, и только после этого втягивается воздух в трахеи. Через дыхальцы от 2-го до 6-го брюшного сегмента термографом записана относительно слабая экспирация и сильная инспирация, а через дыхальцы 7-го брюшного сегмента часто отмечена только сильная экспирация без инспирации. Путем записи детектора теплопроводности (катарометра) и записи инфракрасного газового анализатора установлено, что между «выхлопами» CO_2 выделение этого газа является незначительным.

Периодические волны сокращения брюшных мышц происходят независимо от циклов газообмена и лишь более значительные сокращения являются активными дыхательными движениями и в тот период диффузноконвекционные циклы газообмена могут исчезать. По типу ВГ куколки ПО четко разделяются на три группы: *А* — с ясными циклами ВГ, *Б* — с беспорядочным ритмом ВГ и, *В* — с ациклическим ВГ без проявления активной и пассивной вентиляций. Полагается, что у особей ПО с ациклическим и постоянным ВГ главную роль в ВГ играет чистая диффузия, а у особей с ярко выраженным циклическим ВГ — конвекция.