Oscillating Haemolymph 'Circulation' and Discontinuous Tracheal Ventilation in the Giant Silk Moth *Attacus atlas* L.

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Summary. 1. In the moth *Attacus atlas* (Saturniidae) an oscillating haemolymph 'circulation' and its coordination with tracheal ventilation are described. Periodic heartbeat reversal, intermittent backward haemolymph flow through the perineural sinus and two different superimposed modes of abdominal movements are analyzed by means of contact thermography and photocell measurements.

2. Intraperiodic fluctuations and age dependent alterations in heartbeat frequency and duration of pulse periods are discussed with respect to changes in haemolymph volume and haemocoele capacity.

3. The frontal aortal sac shows transport activity only during the forward pulse period of the heart; during the backward pulse period the amount of haemolymph in the head is reduced. The aorta continues to pulse in the freshly severed head.

4. The expiratory air flow at the spiracles and spiracular valve closing were investigated. In the anterior part of the body expiration occurs slowly as a consequence of haemolymph accumulation during the forward pulse period of the heart, while inspiration takes place as a consequence of removal of haemolymph from the anterior body into the abdomen during a backward pulse period. When most haemolymph is accumulated in the abdomen, expiration of the abdominal tracheal system is accomplished by bouts of abdominal peristaltic movements. The latter are aided by coordinated closing of the abdominal spiracular valves.

5. Transient haemolymph pressure increase by ventilatory movements is probably restricted to the abdomen by a septum and valve in the anterior abdomen. This compartmentation of the adult lepidopteran body combined with haemolymph oscillation is suggested to be a principle advantage in optimal utilization of a small haemolymph quantity with regard to tracheal ventilation in flight-adapted, lightweight construction.

Introduction

In the open 'circulatory' system of insects an interrelation between haemolymph pressure or volume and intratracheal pressure is generally acknowledged, but the effects of haemolymph transport upon tracheal ventilation have not been examined. On the other hand, no one has studied the influence of ventilatory movements upon haemolymph flows and their effects in mediating pressure to the tracheae of different parts of the body. While such ventilatory movements have been observed in large and active insects, it is assumed that in small or inactive insects gas exchange takes place by diffusion alone (Krogh 1920; Wigglesworth 1972). In resting *Papilio machaon* abdominal ventilatory movements occur regularly but in discontinuous bouts (Wasserthal 1980). These 'volleylike' ventilatory movements resemble the patterns of discontinuous ventilation or cyclic CO_2 release described in larger resting insects (review by Miller 1974).

In resting *Papilio*, haemolymph is periodically withdrawn from the thorax and accumulated in the abdomen by coordinated backward haemolymph streaming through the perineural sinus and through the backward pumping heart (Wasserthal 1980). The bouts of ventilatory movements in this species always coincide with the phase of lowest haemolymph amount within the abdomen. It has been suggested that the slow oscillations of haemolymph within the insect body aid a slow periodic tracheal ventilation, especially of the anterior part of the body, while the bouts of abdominal ventilatory movements would affect only the abdominal tracheal system. However, in these relatively small insects this relation could

not be directly tested. Since, in large saturniid moths, heartbeat reversals were very regularly and precisely coordinated with pulsatory activity of the accessory tergal organs (Wasserthal 1976, 1978), it seemed probable that an oscillating haemolymph supply would also occur in representatives of this lepidopteran superfamily. In addition to a comparative analysis of heartbeat periodicity and streaming of haemolymph within the perineural sinus relative to abdominal movements, these large moths can also be used to simultaneously record haemolymph flow in the head and air flow through the spiracles. Since adult giant silk moths rest calmly under daylight with wings open and are unable to feed, they lend themselves to uninterrupted measurements over extended periods of time for examination of age dependent phenomena without narcosis.

Materials and Methods

Animals. The giant silk moth *Attacus atlas* L. was obtained as pupae from Taiwan and the Philipines and their offspring bred in a climatized room on *Syringa vulgaris* (Oleaceae). The moths were in a good condition for 14 days after eclosion at 20 to 23 °C and 90% R.H. During the experiments the unanaesthetized moths were resting in a natural position on the cocoons under these climatic conditions.

Methods. The pulsations of the anterior and posterior portions of the heart and the flow patterns of the frontal and the perineural sinuses were analyzed by contact thermography (Wasserthal 1980). With 'hot' thermistors ($\Delta \vartheta$ 1.7 to 1.8 °C; 'C-method') placed on the cuticle, the convective and conductive cooling effects of haemolymph below the thermistor site were recorded (Fig. 1). With 'cool' thermistors ($\Delta \vartheta$ 0.35 ± 0.05 °C; 'T-method') the metabolic heat of the moths was measured and the direction of air flow through the spiracles analyzed. The direction of haemolymph pulses and haemolymph flow in the abdomen was determined by the T-method after elevating the temperature of the thoracic haemolymph by irradiating the dorsum of the thorax to a $\Delta \vartheta$ of 0.5 °C with a fibre glass lamp (Schott KL 150) from a distance of 12 to 15 mm. The scales were removed from the cuticle and the thermistors fixed in place on pharate adults during the last hours before emergence and just after emergence, when the moths were least reactive to handling. Hence, narcosis could entirely be avoided. For recording of the air flow at the spiracles the thermistor was arranged so that its free bead was 0.3 to 0.5 mm distant from the centre of the spiracle. The thermistor shaft was either stuck directly onto the cuticle near the posterior abdominal spiracle or fixed onto a micromanipulator beside the metathoracic spiracle. The technical details of contact thermography are dealt with in a previous paper (Wasserthal 1980).

The metabolic heat in the inner abdominal haemocoele of resting *A. atlas* is about 0.5 °C above an ambient temperature of 21 °C at 90% R.H.. Although small warming pulses may be registered with 'cool' thermistors upon the heart, the C-method was applied for better pulse visualization; the convective cooling effect upon the heated thermistor measuring sites by haemolymph pulses passing below it is so dominant that the slight differences of metabolically-warmed haemolymph do not significantly interfere with it.

The photocell apparatus for measurement of changes in abdominal length and the techniques for anatomical and histological
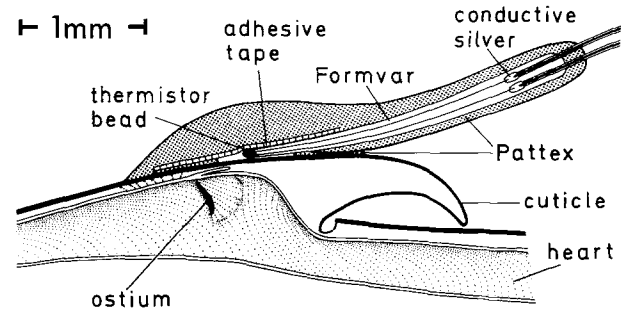

Fig. 1. Arrangement of a thermistor at a posterior tergite above a heart segment. The longitudinal section shows the right heart ostium. Proportions of probe and heart to scale

preparations are identical with those described in connection with *P. machaon* (Wasserthal 1980). The movements of the abdomen were additionally analyzed by means of cinematography (Beaulieu R 16; 24 frames·s^{-1} and time lapse 2 and 0.4 frames·s^{-1}).

Results

General Anatomy of the Saturniid Circulatory System

The abdominal heart is a tube consisting of 8 (in males) or 7 (in females) chambers, each with a pair of dorso-lateral ostia (Fig. 1). While the second to the last chambers of the heart are closely attached under the tergites, the first heart chamber lies further from the cuticle of the first abdominal tergite (Fig. 2). The second heart chamber has a much wider lumen (maximal diameter 1,400 µm) than the other heart chambers (maximal diameter of 6th segment 700 µm). In the anterior part of the body, the dorsal vessel continues as an aorta with a characteristic loop in the mesothorax. The aorta possesses one pair of ostia in both the mesothorax and the metathorax. These ostia connect the aorta directly or indirectly with unpaired accessory pulsatile organs. In the head the aorta passes between esophagus and brain and widens anteriorly into a frontal sac which is provided with a pair of antennal arteries and a pair of funnel-shaped arteries terminating near the eyes, both with openings for haemolymph outflow. The general organization of the dorsal vessel is similar to that of *Bombyx* (Gerould 1938) and *Sphinx* (Brocher 1920).

The perineural sinus (PNS) is regarded as the other main haemolymph passage in the insect body (Jones 1977). It is separated from the perivisceral sinus by the ventral diaphragm (Fig. 3) which in Lepidoptera is attached to the ventral nerve cord (Fig. 3 b) (Richards 1963). In *A. atlas* the anterior abdominal section of the median connective tissue component (VD) of the ventral diaphragm is enlarged (Fig. 3 b).
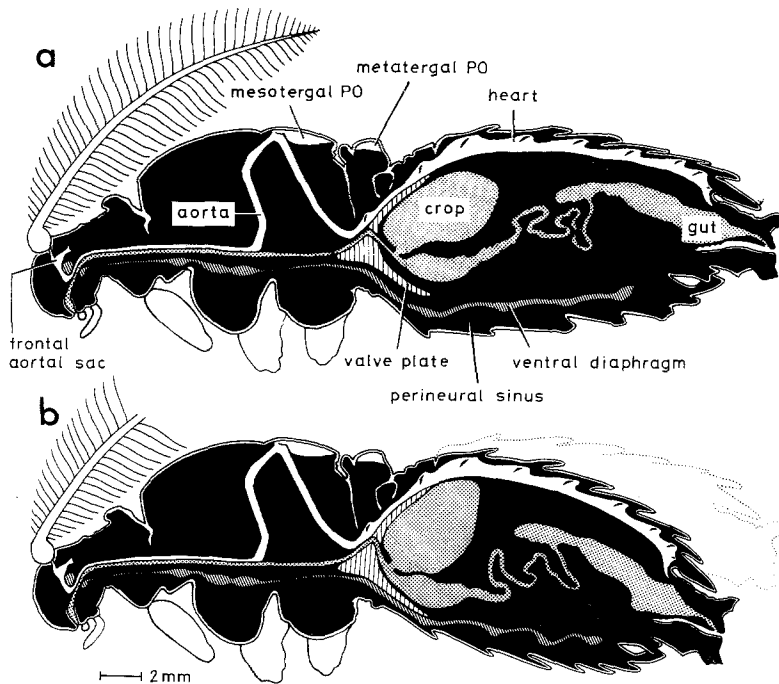
Fig. 2a, b. Location of the organs essential for haemolymph transport in *Attacus atlas* (drawn from a 300 μm polished longitudinal section).
a Abdomen expanded and valve plate opened as during heart pause.
b Abdomen contracted and valve plate closed as during the first half of forward pulse period of the heart. Change in abdominal contour is based upon time lapse cinematography

A dense and solid plate of flattened fat cells with a thickness of 50 to 150 μm lies loosely on top of the ventral diaphragm (Figs. 3, 4: VP). Caudally this horizontal fat body plate terminates at the level of the 3rd abdominal sternite. The lateral parts of this plate extend more posteriorly and seal the lateral spaces next to the muscle insertion of the ventral diaphragm. Anteriorly this fat body plate rises to form a vertical fat layer between the first and second abdominal segments (Figs. 2, 4: VS), sealing the rear of the transverse muscle septum (Hessel 1969). It is remarkable that even in 2 to 3 weeks old moths (which have consumed much of their fat tissue) this fat body plate and vertical septum are still present. It is assumed that both the enlarged ventral diaphragm and the horizontal fat body plate are parts of the valve mechanism for periodic separation of the haemocoele of the anterior body from the abdomen, as in *P. machaon* (Wasserthal 1980). The tightness of this specialized fat tissue is demonstrated by osmium distribution after injection of 1% OsO_4 fixative into the abdominal perivisceral cavity (Fig. 3b). By fixing the valve region under pressure via the thorax the valve plate remains in the upward position away from the ventral diaphragm (Fig. 4). Further observations show that the principal morphological constituents of the valve are common among Lepidoptera, and are also present in Sphingidae, Pyralidae, Pieridae and Brassolidae. Accordingly the same mode of operation can be assumed.

Periodic Heartbeat Reversal

By means of simultaneous recordings of the pulses at an anterior heart chamber (thermistor on the surface of the 2nd abdominal tergite) and at one of the posterior heart chambers (6th, 7th or 8th abdominal tergite; $n=15$ specimens), a periodic change of the metachrony of heart pulses could be demonstrated (convective cooling effects (=downstrokes) in the thermographs of Fig.5). By elevating the temperature of the thoracic haemolymph, periods of warming pulses alternating with periods of cooling pulses were detected with a single 'cool' thermistor at the heart (2nd tergite; cf. Fig. 10, $n=3$). The warming pulses represent the backward pulsations of the dorsal vessel.

During the shift from forward to backward beating of the dorsal vessel, the heart generally stops beating for an average duration of 9 sec at 22 °C ($0.15 \min \pm 0.1$; $n=12$). At the end of the forward pulse period incomplete peristaltic waves are frequently observed. A similar phenomenon has been observed in *P. machaon* (Wasserthal 1980). Such irregularities were more abundant in aged, stressed or less vital specimens of *Attacus*. As a result of incomplete peristaltic heart contractions, the duration of pulse periods and pauses may differ, according to the different regions of the heart. For evaluation of pulse rate and period length (below, Figs. 7, 8) the thermographs of the posterior heart segments were
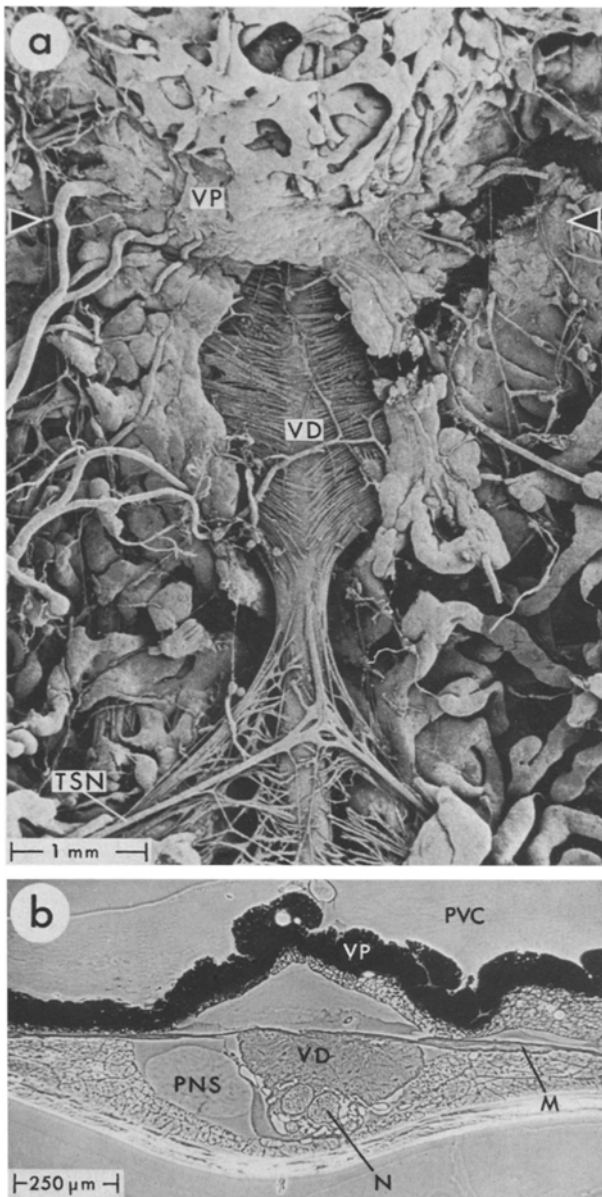
Fig. 4. Longitudinal section through the valve region with the horizontal fat body valve plate (*VP*) lifted upward in the open position, leaving a haemolymph-filled space above the ventral diaphragm (*VD*). *PNS* perineural sinus; *VS* vertical fat body septum, *1, 2, 3* first, second, and third abdominal sternite; *arrowhead* indicates position of PNS-thermistor

Fig. 3a, b. Ventral diaphragm (*VD*) and valve plate (*VP*) in the anterior abdomen. a Dorsal view, SEM. *Arrowheads* indicate level of section in b. *TSN* transverse segmental nerve. b Cross section of ventral diaphragm with enlarged connective tissue ledge (*VD*), ventral nerve cord (*N*), and muscles (*M*). The valve plate (*VP*) is osmified only from the perivisceral cavity (*PVC*). *PNS* perineural sinus. Phase contrast micrograph

used, the pauses being defined as parts of the reverse pulse periods.

Comparison of Single Pulses at the Anterior and Posterior Heart (C-Method)

The convective cooling effect of a single propulsion of haemolymph through a heart chamber is characteristically different at the anterior and posterior chambers of the heart (Fig. 5). The amplitudes of
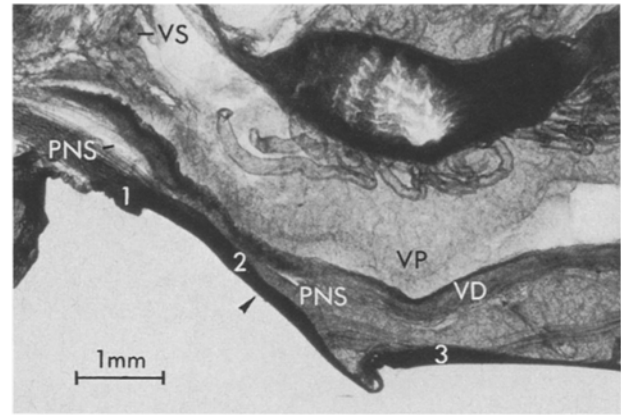
backward pulsations of the heart are significantly greater at the 7th than at the 2nd heart chamber, thus suggesting a higher haemolymph volume per pulsation (Figs. 6, 7). Within the 2nd heart segment, however, the backward pulse consists of two consecutive convective effects (numbered with 2 and 5 in Fig. 5b) with a short cessation of cooling or even rewarming between these two events, while at the posterior end of the heart the backward pulse manifests itself as a single convective cooling phase (numbered with 6 in Fig. 5b). Comparison of pulse amplitudes as a means of estimating the relative pulse volume in different parts of the heart is of debatable value because of such differences and also because of the differences in diameter of the various heart chambers.

Within a given posterior heart chamber, the amplitudes of the reverse pulses are significantly greater than the amplitudes of the forward pulses (Fig. 7). The greater amplitudes of the reverse pulses could reflect a greater haemolymph volume per pulse, but this may not necessarily be the case because of the slower pulse rate (cf. Wasserthal 1980).

A short, generally inconspicuous cessation of rewarming at the beginning of diastole can be observed during forward beating at both the posterior and anterior heart chambers, but during backward beating this occurs at the posterior heart segments only (Fig. 5, arrowheads). This convective effect may be ascribed to the action of the ostial valves. Within the 7th heart chamber the cessation of rewarming at diastole during forward beating is followed by another small but more distinct convective cooling (Fig. 5a, asterisk). It cannot yet be decided whether this is due to the activity of the heart tube or to a contraction of the alary muscles.
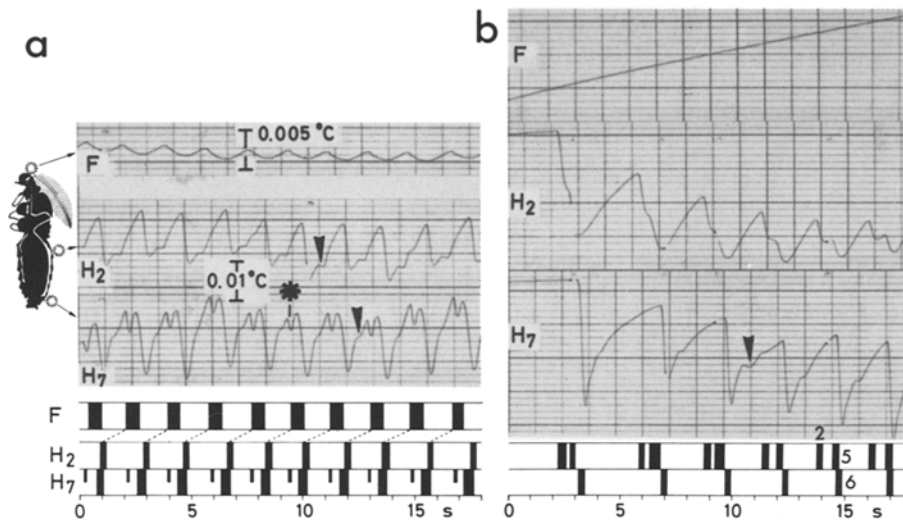
Fig. 5a, b. Analysis of forward pulses (**a**) and reverse pulses (**b**) of the heart in a female, 5 days old (C-method).
In **a** the metachrony of downstrokes (*black bars* above the time scale) runs from the posterior heart (H_7) to the anterior heart (H_2) and the frontal aorta (F);
in **b** the downstrokes of H_2 precede those from H_7, no pulses being perceptible at F.
Arrowheads indicate convective effects probably due to contraction of the ostial valves, *asterisk* see text
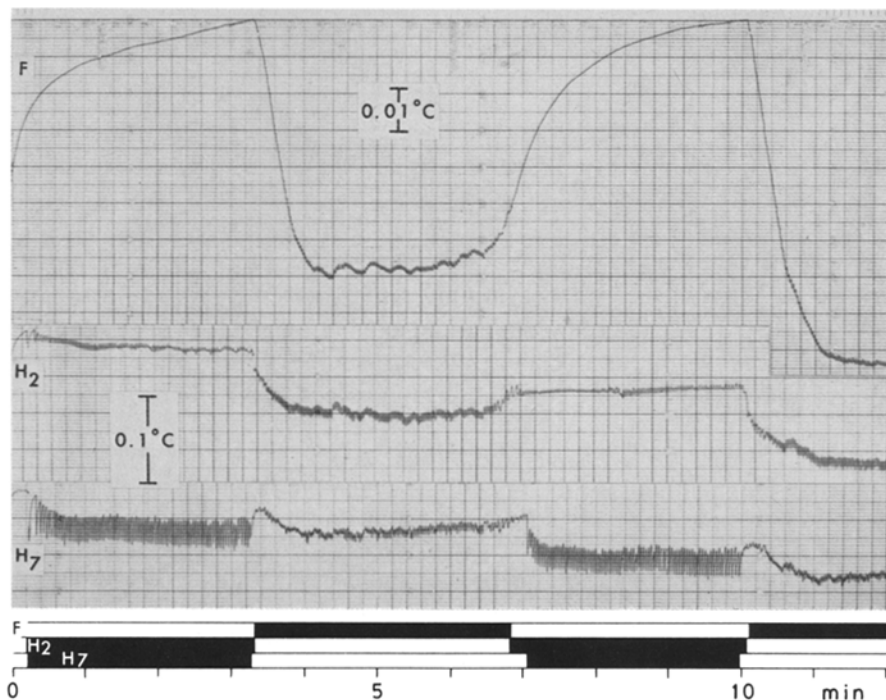

Fig. 6. Coordination of heart periodicity and intermittent transport activity of the frontal aortal sac (F). Survey of Fig. 5; C-method. Periodic changes of the temperature level upon the heart segments (H_2, H_7) are caused by different convective cooling. Temperature increase upon F during the reverse pulse periods is a consequence of reduced thermal conduction due to reduced haemolymph content in the head. *Black bars* in F pulse period, in H reverse pulse period

Fluctuations of Pulse Rate, Pulse Amplitude and Alteration of the Envelope Curve of the Heart (C-Method) and Its Implications for Transported Haemolymph Volume

During each period of forward and backward pulsations typical fluctuations of heartbeat frequency and pulse amplitude were recorded (Fig. 7). The rate of forward beating is generally highest at the beginning of the periods and lowest at their ends. Since the pulse amplitudes also decrease toward the end of the

forward pulse period at the anterior and posterior end of the heart, a reduction in the amount of transported haemolymph in *Attacus* is assumed similar to the events observed in *Papilio* (Wasserthal 1980). This is consistent with the rising level of the envelope curve (obtained by connecting the maxima of all pulses) at the end of the forward pulse period, which reflects a diminished average convective effect of the last forward pulses. A similar reduced haemolymph transport volume during the last backward pulses may also be deduced from the decreasing amplitudes and
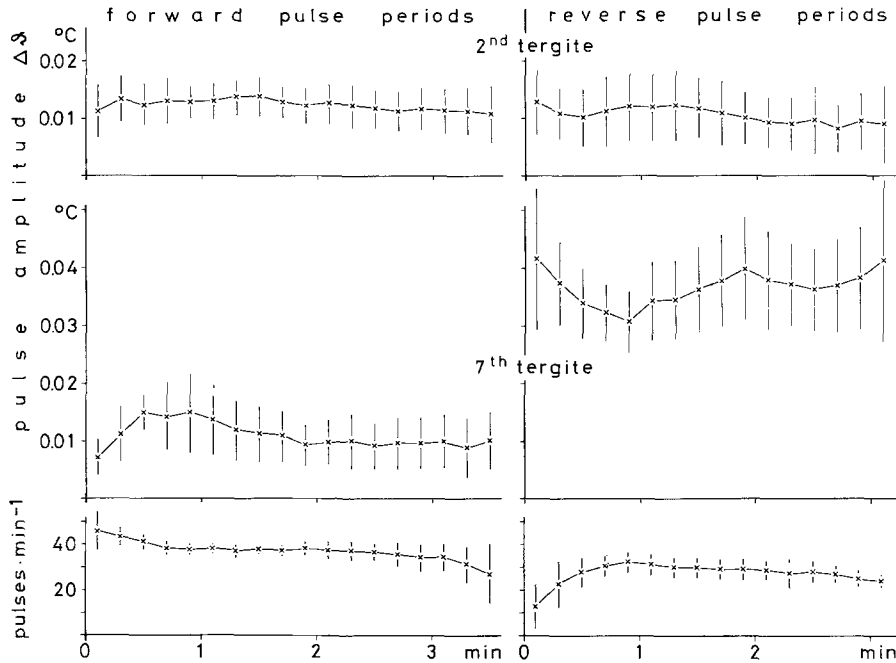
Fig. 7. Fluctuations of heartbeat frequency and amplitude in the course of single pulse periods at the anterior and posterior heart. $n=6$ females (22 °C)
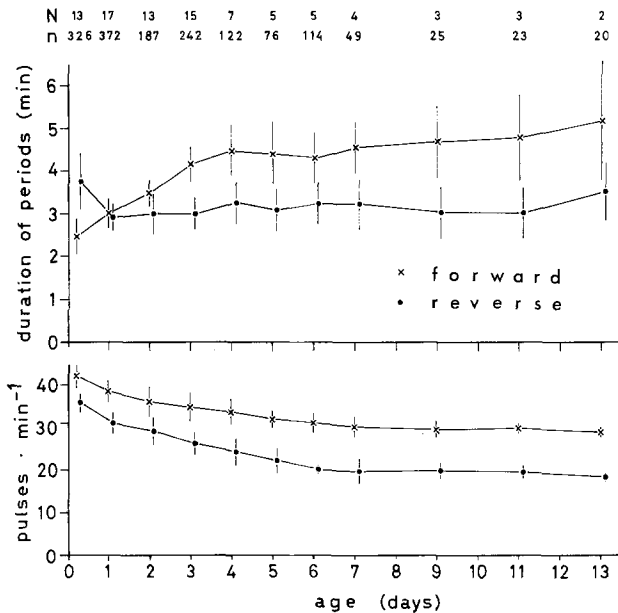

Fig. 8. Heartbeat frequency and period length throughout adult lifetime at 22 °C. N number of specimens, n number of periods

rate of the last reverse pulses combined with an increasing level of the envelope curve of the 2nd heart chamber and frequently of the posterior heart chambers. The fluctuations of the pulse amplitude at the posterior end of the heart are typically almost inversely proportional to the pulse rate. Therefore, no decision about change or constancy of haemolymph transport can be made here.

Age Dependence of Pulse Rate and Duration of Heartbeat Periods

The average rate of forward pulses is from 8 to $10 \cdot \mathrm{min}^{-1}$ higher than the rate of backward pulses. Both forward and backward pulse rates decrease during the lifetime of the adult *A. atlas* from an initial frequency of 42 pulses $\cdot \mathrm{min}^{-1}$ (forward) and $36 \cdot \mathrm{min}^{-1}$ (backward), 6 h after emergence, to 30 pulses $\cdot \mathrm{min}^{-1}$ and $18 \cdot \mathrm{min}^{-1}$, respectively, after about two weeks (Fig. 8). During the 3rd to 12th h after eclosion, the reverse pulse periods are markedly longer than the forward periods, yet within the first day of adult life, the reverse pulse periods become shorter and the duration of forward pulse periods increases. 24 h after eclosion until senility the duration of the reverse pulse periods remains constant (about 3 min), while the forward pulse periods do not become constant before the 3rd day of adult life. Then, forward pulse periods are 1.4 to 1.5 times longer than the reverse pulse periods.

Intermittent Transport Activity of the Frontal Aortal Sac and Periodic Change of the Haemolymph Volume in the Head (C-Method)

With a thermistor attached medially at the base of the antennae of intact moths ($n=2$), pulses could be detected only during forward beating of the dorsal vessel (Figs. 5, 6). The pulse frequency is identical with that of the heart, but the pulse amplitude of the frontal sinus is less than 1/10 that of the heart.
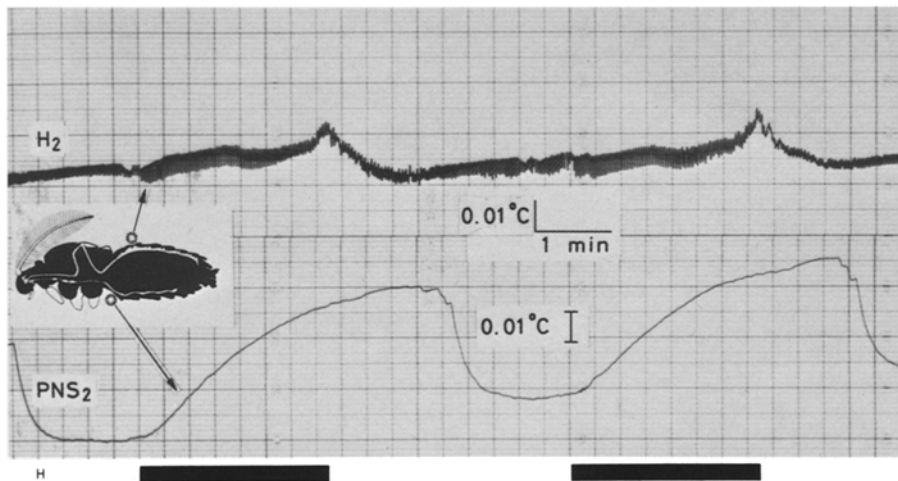
Fig. 9. Periodic change in thermal convection at the perineural sinus (PNS_2) and its coordination with heart periodicity (H_2) in a female, 1 d old (C-method). *Black bar*: reverse pulse period of the heart

The interval between the main convective cooling at the 7th heart chamber and the convective cooling at the front is about 1.5 s. The cephalic part of the aorta is able to continue pulsating in a severed head. During backward beating of the heart no pulses of the cephalic aorta are recognizable at the front. Instead there is a rapid temperature increase, beginning during the heart pause and continuing until the end of the reverse pulse period without achieving a constant level. During the first forward pulses, the temperature falls to a lower level again. The cooling rate at the beginning of the forward pulse period is higher than the rewarming rate at the beginning of the reverse pulse period (Fig. 6).

Conclusions. The recorded pulsations in the head during forward beating of the dorsal vessel are attributed to the activity of the frontal aortal sac which pulsates in exact metachrony with the forward beating heart in the abdomen. The small amplitude of the forward pulses at the front end indicates that convective cooling effects are damped, probably by the thick head cuticle and the relatively great distance (about 1 mm) of the frontal sac from the head surface. The complete lack of single convective cooling effects in the head during backward beating of the heart may be due to an extreme reduction of the pulse volume or may be due to a pause in the contractions of the frontal sac.

The steep and relatively high temperature increase at the front end of the moth, without reaching a constant level within 3 min of the beginning of a reverse pulse period, must be caused by a reduction of thermal conduction during this time. The difference of the average temperature levels, between forward pulse and backward pulse periods at the front, is too great to be merely a consequence of the different pulsation capacity of the frontal aortal sac. This, and

the continuous (and not pulsewise) decline of thermal conduction, points to a decrease in amount of haemolymph in the head and indicates that this decrease is brought about by a different mechanism than contractions of the dorsal vessel. According to the different rate of cooling and rewarming, respectively, it can be concluded that the process of haemolymph removal from the head is slower than its renewed influx into the head.

Periodic Haemolymph Transport in the Perineural Sinus (PNS)

Thermistor recordings from the anterior PNS (C- and T-method; measurements at the 2nd sternite, $n=5$) show slow changes in temperature which coincide with forward and backward pulse periods of the dorsal vessel. At about the middle of the forward pulse period, the temperature drops in the C-curves and gradually rises after the onset of the reverse pulse period of the heart (Fig. 9). After heating the thoracic haemolymph, the T-curves at the PNS show an inverse form in comparison with the C-curves: Instead of a sudden temperature drop in the C-curves there is a temperature increase in the T-curves (Fig. 10).

In dissected specimens of *A. atlas* the ventral diaphragm can be observed to push the haemolymph backwards thrashing with a frequency of about 1 to 2 pulses $\cdot$ s^{-1}. No pulse effects of a comparable frequency could be detected in the PNS-curves. Neither phases of inactivity nor any coordination of the thrashing frequency with that of other pumping organs could be recognized.

Conclusions. The lack of pulse effects of the ventral diaphragm in the PNS-curves supports the assumption that this organ is of secondary significance for haemolymph transport from the thorax into the abdo-
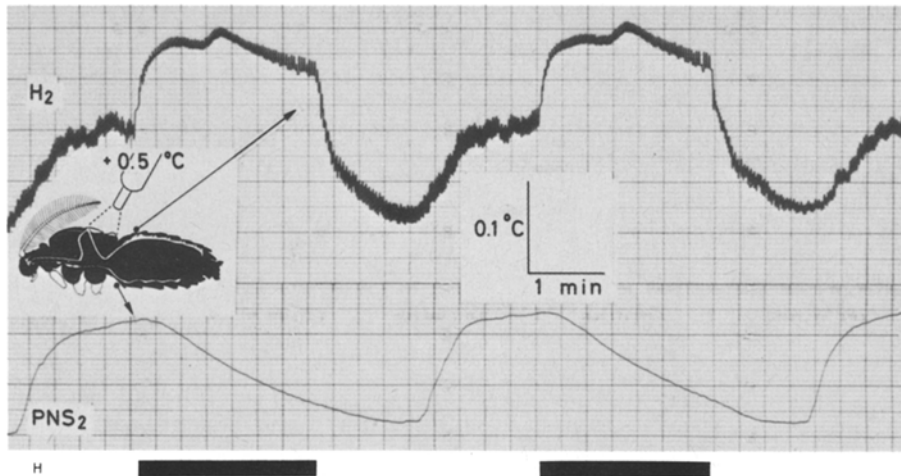
Fig. 10. Periodic backflow of heat-marked thoracic haemolymph through the abdominal perineural sinus (PNS_2) beginning after the first half of the forward pulse period of the heart (H_2) in a female, 1 d old (T-method). The periodic haemolymph backflow through the PNS affects also the temperature of the heart. *Black bar*: reverse pulse period of the heart
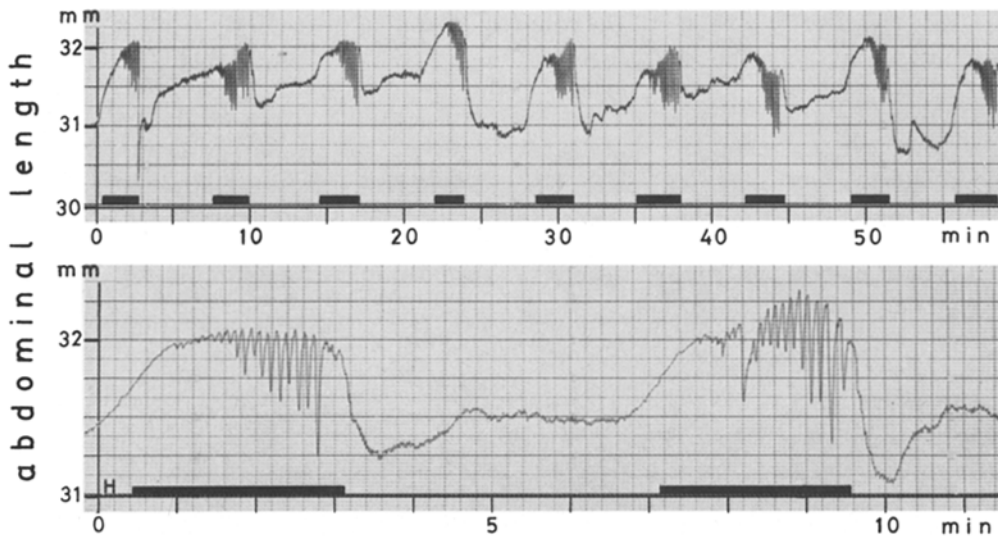

Fig. 11. Periodic changes in abdominal length and volleylike abdominal movements and their coordination with heartbeat periodicity in a female, 2 d old (Si-photocell measurement). *Black bars*: reverse pulse periods of the heart

men and mainly serves to distribute the haemolymph within the abdomen. The backward haemolymph transport through the PNS manifests itself as a single extended flow period in the course of each heartbeat sequence (=forward pulse period+pause+reverse pulse period): Transport begins at the middle of the forward pulse period and lasts until the end of the reverse pulse period of the dorsal vessel. According to the coincidence of the most effective convective cooling (in the C-curves) with the arrival of maximally heated thoracic haemolymph (in the T-curves), the flow intensity of haemolymph is assumed to be highest during the last third of the forward pulse period until the beginning of the reverse pulse period of the dorsal vessel. The flow through the PNS gradually declines during the course of the reverse pulse period and is lowest or may cease during the first half of the following forward pulse period of the heart.

*Periodic Changes in Abdominal Length
and Ventilatory Movements
Coordinated with Heartbeat Periodicity*

By means of photocell recordings and 16 mm time lapse cinematography, periodic changes in abdominal length were recorded in male and female *Attacus* throughout adult lifetime (Fig. 11). Two types of changes in abdominal length occur in *Attacus*. Type 1: A slow shortening and lengthening of the abdomen takes place during the course of one heartbeat sequence. Type 2: Bouts of peristaltic movements over the abdomen are performed during backward beating of the heart.

Type 1. At the beginning of the forward pulse period of the heart, the abdomen shortens and reaches its minimum length. This is followed by two phases of
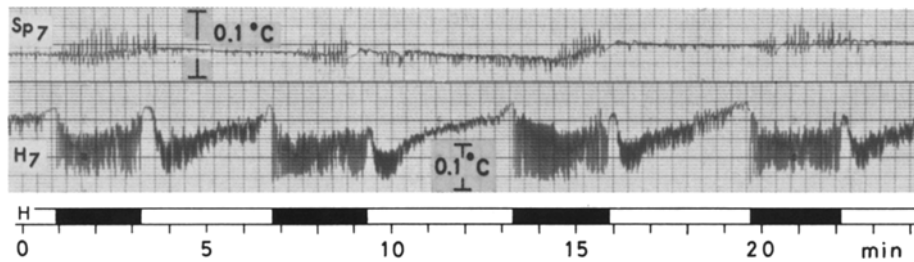
Fig. 12. Air temperature in front of the posterior abdominal spiracle (SP_7). Volleys of expired (warm) air pulses during reverse pulse periods of the heart (H_7). Female, 3 d old (T-method). *Black bars*: reverse pulse periods of the heart

abdominal lengthening: The first begins after about one-fourth to one-third of the forward pulse period. The second, more conspicuous lengthening, is initiated at the end of the forward pulse period, the movement being most vigorous during each heart pause and continuing during the reverse pulse period of the heart until the end of its backward beating. The total change of abdominal length is 1 to 2 mm. During the following slow contraction, the abdominal segments are telescoped, especially along the ventral side, while their diameter may decrease (in males) or remain constant (in females) (Fig. 2b).

Type 2. The peristaltic abdominal movements have a frequency of 6 to $10 \cdot \mathrm{min}^{-1}$ at 22 °C. Generally they do not begin before half a minute after onset of the reverse pulse period of the heart. The amplitude of these movements generally increases in the course of one bout. Sometimes these 'volleylike' movements do not stop exactly at the end of the reverse pulse period, but one or two contractions may occur after the onset of forward beating of the heart. These peristaltic movements were not performed when the moths were disturbed. Each peristaltic wave begins at the caudal end of the abdomen and proceeds anteriorly. It leads to a reduction of the diameter of the segments involved and results in a stretching of the intersegmental folds. The measured 'shortening' of the abdomen is somewhat misleading, since it results from a slight ventral bending of the abdominal tip with each peristaltic wave.

Coordination of Spiracular Activity with Peristaltic Ventilatory Movements of the Abdomen

During recording of heartbeat periodicity the closing of the (meta-)thoracic spiracular valve and of the abdominal spiracles in segments 4 to 7 were observed with a stereomicroscope after removing the scales from the surrounding cuticle.

The metathoracic spiracular valve was never observed to be closed throughout any complete sequence of forward and reverse heartbeat periods; most frequently it was opened widely, although sometimes it was seen 'fluttering'. However, the abdominal spiracles close when the corresponding segment contracts

in the course of each peristaltic wave of the 'volleylike' abdominal movements. Generally, the spiracles close with some delay following the onset of the contraction of the respective segment, but they open exactly at the onset of the segmental relaxation. The abdominal spiracles close metachronously, beginning at the posterior segment and continuing anteriorly along with the abdominal peristaltic contraction. During the slow abdominal shortening at the beginning of the forward pulse period of the heart, all the spiracles remain open. This phase is followed by 'fluttering' movements until the next ventilatory phase. Thus no phase of spiracular constriction was observed after the bouts of peristaltic movements.

Expiratory Air Flow at the Spiracles (T-Method)

Abdominal Spiracles

To answer the question whether air is expired during the slow abdominal shortening or during the bouts of peristaltic movements, respiratory air flow was recorded by temperature measurements.

In two specimens a thermistor was fixed to the 7th abdominal spiracle or positioned next to the metathoracic spiracle, with the thermistor bead exposed freely to the air stream leaving and entering the spiracle. While the heartbeat periodicity was simultaneously recorded, the beginning and duration of the abdominal contraction was observed visually and plotted on the recording paper by a hand-controlled event marker.

Since the body temperature of resting *A. atlas* lies above ambient and slightly exceeds the thermistor temperature during T-measurements, expiration results in a temperature increase at the thermistor bead. During the slow abdominal shortening (Type 1) at the beginning of the forward pulse period, no warmed air passes over the abdominal spiracular thermistor bead (Fig. 12). During the bouts of abdominal movements, however, a pulse of warm air leaves the abdominal spiracle at the beginning of each peristaltic contraction. This pulse is followed by an abrupt cooling which lasts until the end of each contraction (Fig. 13). In the pause between two peristaltic movements a slight rewarming takes place. Small irregular undulations were superimposed on all phases of the temperature curve. They seem to correspond to the observed spiracular 'fluttering' movements.
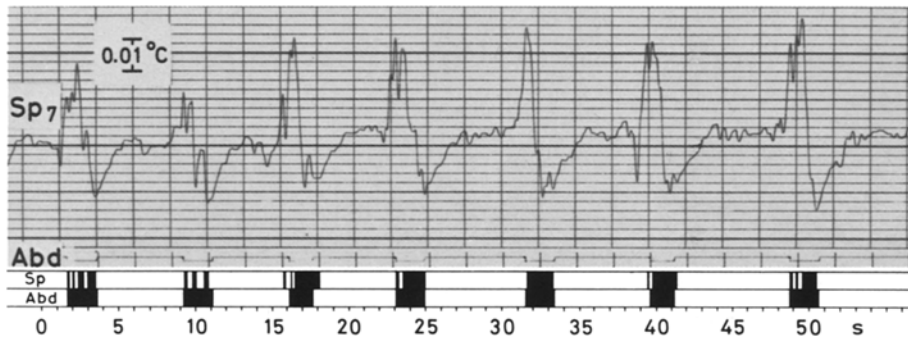
Fig. 13. Air temperature in front of the posterior abdominal spiracle (Sp_7). Warm expiratory air pulses are caused by volleylike abdominal contractions (*black bars* in *Abd*). Closing of the spiracle valve (*black bars* in *Sp*) interrupts expiration and cools the thermistor to ambient temperature. Female, 3 d old (T-method)
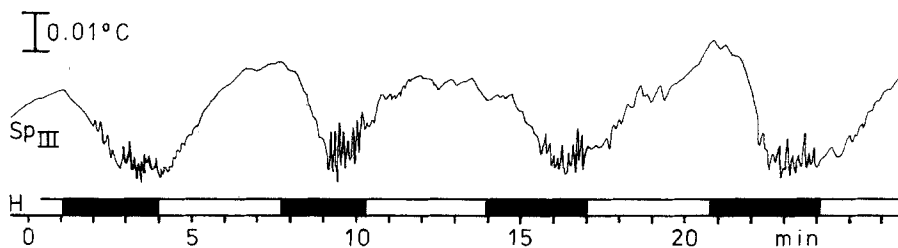

Fig. 14. Periodic change of air temperature in front of the open metathoracic spiracle (Sp_{III}) (T-method). The warming during forward beating of the heart and the warming pulses during reverse beating (*black bar* in *H*) indicate expiration of tracheal air

Conclusions. The single, slow but nevertheless pronounced abdominal shortening at the beginning of the heart's forward pulse period does not result in expiration through the posterior abdominal spiracles, and thus does not appear to compress the abdominal tracheae. Each contraction during a series of peristaltic movements, however, causes an expiratory air flow. The air flow at the spiracle thermistor is influenced by the closing and opening of the spiracular valve. The temperature drop which immediately follows the streaming of warm air is caused by the closing of the spiracular valve, interrupting the expiratory air flow when the abdominal contraction of the segment involved is most vigorous. The slight rise in temperature during opening of the abdominal spiracular valves may be caused by an outward diffusion of warmer intratracheal water vapour against the inspiratory air stream and/or by heat radiation from the unscreened tracheal lumen. While the anterograde progression of the ventilatory peristalsis and the synchronized but delayed closing of the abdominal spiracular valves brings about an expiratory air flow directed from the tracheal trunks of the posterior abdominal segments to those of the more anterior segments, the inspiratory air enters the abdominal tracheae at the spiracles of all segments equally.

Metathoracic Spiracles

In contrast to the mean temperature course at the abdominal spiracle, the air temperature at the thoracic spiracle varies in correlation with the change in the heart's beating direction (Fig. 14). Warm air

passes by the thermistor bead during each forward pulse period of the heart, whereas during heart pause and the reverse pulse period the thermistor cools again. During the second half of the reverse pulse period, however, pulses of warmed air are superimposed on the recooling phase. The superimposed pulses are synchronous with the 'volleylike' abdominal ventilatory movements. While the temperature increase during the heart's forward pulse period reaches 0.03 °C, the temperature amplitude of the air pulses is less than 0.01 °C. Additional short irregularities in cooling or warming rate of the slow temperature changes were often observed to coincide with the spiracular valve 'fluttering'.

Conclusions. The small but distinct expiratory pulses at the metathoracic spiracle show that the stream of expiratory air caused by the abdominal ventilatory movements is really directed anteriorly. The approximately three-fold temperature increase during the extended expiratory phase demonstrates that thoracic air is expired mainly during the forward pulse period of the heart and inspired during the reverse pulse period.

Discussion

Coordination of Periodic Heartbeat Reversal and Ventral Haemolymph Transport with Abdominal Movements

In the moth *A. atlas*, haemolymph is slowly moved back and forth within the body as in the butterfly
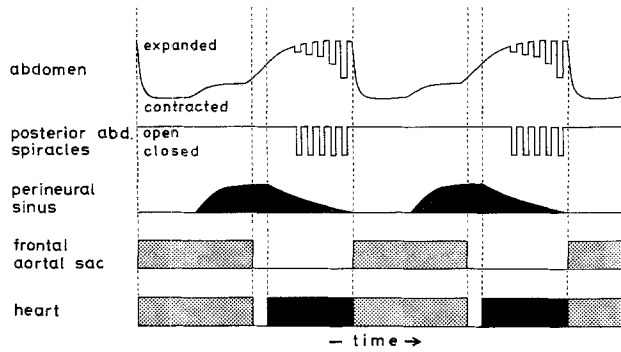
Fig. 15. Coordination of abdominal activity, spiracle closing and haemolymph transport periodicity in *Attacus atlas*. *Open*: pauses of transport activity; *shaded*: forward transport; *black*: backward transport

P. machaon (Wasserthal 1980). This oscillating haemolymph supply is brought about by periodic heartbeat reversal, heart pauses, and intermittent flow through the PNS. All these events are coordinated with periodic abdominal movements (Fig. 15) but the timing and duration of each event is characteristically different: In the moth, the heart pauses at the end of the forward pulse periods are shorter, but the backward pulse periods are conspicuously longer than in *P. machaon*. Abdominal lengthening in *A. atlas* occurs in two steps: first by a less marked relaxation phase during the first half of the heart's forward pulse period, and then by a more pronounced expansion, which begins at the end of the forward pulse period. This second lengthening step in *Attacus* thus occurs at the same time as the single abdominal length increase in *P. machaon*. The abdominal relaxation is believed to be responsible for the (antecedent) onset and the relatively long duration of the backward haemolymph flow through the PNS in *A. atlas*. It is remarkable that the onset is so sudden, because the first step in abdominal lengthening continues rather slowly or is even arrested at that time. The sudden onset of backward flow may be due to the fact that when the haemocoele of the thorax is filled by the forward pumping heart, a threshold of thoracic haemolymph volume is exceeded. In addition, the abrupt flow suggests an active opening of the PNS at the anterior end of the abdomen, perhaps by a decrease in volume of the tracheal sacs in the first abdominal segment or by involvement of the transverse muscle septum at the border between the 1st and the 2nd abdominal segment (Hessel 1969). The rather constant length of the abdomen after abdominal relaxation suggests that at this time about as much haemolymph is pumped into the anterior body as is sucked backward into the abdomen. These conditions are similar to the transitory circulatory phase which occurs at the beginning of the heart's forward pulse period in *P.*

machaon, at which time the haemolymph flow pattern approaches that of the circulation model given by Brocher (1931). Displacement of haemolymph from the thorax into the abdomen begins with the second phase of abdominal lengthening, when the volume transported by the forward pulsations of the heart is reduced and thereafter, when the heart stops beating, while the backward flow of haemolymph along the PNS is at a maximum level. The evenness of the backward haemolymph flow in the PNS and of the haemolymph removal from the head suggests a suction effect of the expanding abdomen, whereas the influence of the thrashing ventral diaphragm on backward haemolymph transport is of only minor importance.

Interrelations of Haemolymph Volume, Heart Rate and Duration of Heartbeat Periods

Characteristic changes in the heart rate of adult moths occur generally throughout adult life and also in the course of single forward and reverse pulse periods. It might be asked in which way such changes may be influenced by post-ecdysial events, and whether ontogenetic and periodic changes in haemolymph volume may affect heart rate and period duration. In *A. atlas* the frequencies of both forward and backward pulses decrease during adult life as does the average heart rate in *Bombyx mori* (Masera 1933) and in *Locusta migratoria* (Roussel 1973). The overall frequency reduction can partly be explained as a normal, age-dependent decrease in metabolic activity. In Lepidoptera and Diptera the most significant decline of heart rate occurs, however, in the first few hours of, or on the day after, eclosion (Queinnec and Campan 1972; Wasserthal 1975; Moreau and Lavenseau 1975). This may result from the decreasing influence of a cardio-accelerating peptide which appears to be released during eclosion and wing spreading (Tublitz, personal communication) when the heart frequency is highest. Heart rate may also decrease as a consequence of the rise in haemolymph viscosity due to post-ecdysial water loss (Nicolson 1976). Moreover, post-eclosion diuresis reduces the total haemolymph volume which is accompanied by a significant decline in the average haemolymph pressure (Cottrell 1962; Moreau 1974).

With regard to the abdomen a change in haemolymph pressure probably occurs for short phases during each heartbeat sequence in fully developed *P. machaon* and *A. atlas*. At the beginning of each forward pulse period, when most of the total haemolymph volume is still within the abdomen and the haemolymph pressure would increase due to abdominal shortening, the heart rate is also highest. This

correlation between high heart rate, high abdominal haemolymph content and pressure parallels the situation during wing expansion (Wasserthal 1975; Moreau and Lavenseau 1975). The lowest forward pulse rate, on the other hand, coincides with the time of presumed lowest haemolymph pressure in the abdomen, i.e. when it expands at the end of the period.

The frequency fluctuations in the course of the reverse pulse period are influenced by the intermittent pulse activity of the pulsatile organs in the thorax. This will be discussed in a later study (in preparation).

Maximal forward pulse rate depends on the relation of abdominal haemolymph volume and (variable) tension of the abdominal muscles on the one hand and on the haemolymph content of the anterior body on the other hand. When, at the end of the forward pulse period, the haemolymph content of the head and thorax is highest, not only the pulse rate but also the pulse volume is reduced. This, and especially the standing pulse waves which occur at the end of the forward pulse periods (Wasserthal 1980), suggest that at that time the heart has to operate against a higher pressure of the anterior body. Although back pressure has been postulated as a factor which elicits heartbeat reversal during metamorphosis in some insects (Poyarkoff 1910; Tirelli 1935), it was theoretically excluded with regard to the open insect circulatory system by Richter (1973). However, Jones (1977) considers pressure to be a possible trigger.

Even if the triggering of heartbeat reversal may not be explained merely by back pressure on account of the precise coordination of heartbeat reversal with abdominal muscle activity and on account of heart innervation (Wasserthal 1977), the haemolymph volume and its relation to the haemocoele capacity of the anterior body, especially of the wings, seem to be determining factors for maximal period duration. This is demonstrated by the prolonged forward pulse period during wing spreading. From a large haemolymph reservoir in the abdomen, haemolymph could be pumped forward without interruption until the limit of wing extensibility is reached. After haemolymph has fulfilled its hydraulic function in wing expansion, the excess fluid is transported back into the abdomen by prolonged periods of reverse pulses (Wasserthal 1975). That this does not occur in the same manner within a single long reverse pulse period shows that some additional factor must exist for timing period duration. The definite (reduced) haemocoele capacity in the anterior body is achieved after sclerotization of the exoskeleton and the increase of the tracheal lumen as a response to post-ecdysial water loss (Evans 1935; Behrends 1935; Cottrell 1962) in Attacus about 24 h after eclosion (Wasserthal, in preparation). From this time onwards the duration of reverse pulse periods remains constant. Since the pulse rate still decreases, a further slight reduction in the amount of haemolymph, which is periodically pumped backward, must occur.

It seems that in A. atlas a rather constant haemolymph volume is oscillating between the anterior part of the body and the abdomen. If the pulse rate declines, a longer pulse period is needed to transport a given haemolymph volume and vice versa. This has also been demonstrated in connection with the temperature-dependent changes in heart activity (Wasserthal 1980). The assumption that the duration of heartbeat periods is determined by the haemocoelic capacity of the anterior end of the body rather than by that in the elastic abdomen is in good agreement with the finding that in young blowflies, the duration of the forward pulse period is significantly correlated with the duration of the consecutive reverse pulse periods, but not inversely (Queinnec and Campan 1975).

The similarity of heart activity in Lepidoptera and Diptera suggests that the oscillating haemolymph supply may be a general phenomenon in insects. Fluctuations of heart rate with a minimum at the end of the forward pulse period, the occurrence of heart pauses and a shortening of the reverse pulse period duration throughout the adult lifespan, which are characteristic for P. machaon, have also been found in Calliphora (Queinnec and Campan 1975). As a difference, in female blowflies the forward pulse frequency increases after the 5th day of adult life, and has been related to sexual maturation. The shortening of reverse pulse periods and the loss of a significant correlation between forward and the consecutive reverse pulse periods in the blowfly have also been discussed with regard to abdominal distension during sexual maturation (Queinnec and Campan 1975). In unmated Attacus females with the abdomen visibly distended by eggs neither such an increase in forward pulse rate nor a significant further shortening of the duration of reverse pulse period has been observed.

Interrelation of Oscillating Haemolymph Transport and Tracheal Ventilation

In the anterior end of the body with its rather unelastic exoskeleton the haemocoelic capacity is probably dependent on the elacticity of the tracheal air sacs. The functional interdependence of oscillating haemolymph supply and tracheal ventilation, which was earlier postulated by Wasserthal (1976, 1980), can now be confirmed. A periodic reduction of haemolymph volume within the anterior part of the body is indirectly demonstrated by the reduced thermal conduc-

tion in the head during reverse beating of the heart, and this is correlated with the period of transport inactivity of the frontal sac. The C-curves from the head of *Attacus* are similar to those recorded from the mesothorax of *P. machaon* (Wasserthal 1980). Reduction of thermal conduction in both species indicates an increase in the volume of the tracheal air sacs to compensate for the reduced haemolymph volume. This means that during heart pauses and during the reverse pulse periods of the heart the tracheal system in the anterior part of the body inspires. The expiration during the forward pulse period of the heart is directly documented by records of warm air passing from the metathoracic spiracle. This expiration is shown to exceed significantly the expiratory effects of the anteriorly-directed air pulses caused by the 'volleylike' abdominal ventilatory movements. It is concluded that the ventilation of tracheal air in the anterior end of the body is accomplished gently by the oscillating haemolymph, whereas the 'volleylike' abdominal ventilatory movements mainly affect the abdominal tracheae.

Coordination of Spiracular Activity and Ventilatory Movements

Synchronization of spiracular activity and ventilatory movements has been described in several insect orders (review by Miller 1974). Thus the posterior abdominal spiracles of the grasshoppers *Stenobothrus* and *Tettigonia* and the wingless hippoboscid fly *Melophagus ovinus*, close during abdominal contractions while the thoracic spiracles remain open (Dubuisson 1924a, b; Webb 1945) as they do in *Attacus*. In other species of Saltatoria and Blattodea, however, the abdominal spiracles close during abdominal expansion, while the spiracles of the thorax and first abdominal segments stay open (Lee 1925; Fraenkel 1932; Miller 1974). In some cockroaches, grasshoppers, bees, and mantids the closing pattern of spiracles may change (cf. Miller 1974).

Synchronization of spiracular activity with ventilatory movements has generally been discussed with respect to the production of unidirectional air flow, the functional significance of which may be manifold. A greater efficiency of gas exchange by reducing or abolishing the dead space in the tracheal system (e.g. of the head) has been proposed (Miller 1974). In *A. atlas* the effects of the anteriorly-directed air flow are, however, relatively weak at the thoracic spiracles. Even though some air is expelled here, the thoracic tracheal system is not compressed by the abdominal ventilatory movements, due to the presence of a haemolymph pressure valve behind the waist. It is assumed that by the metachrony of abdominal peristal-

sis with spiracle closing, both air and haemolymph are displaced from the posterior to the anterior end of the abdomen. This process would favour a build-up of pressure for abdominal expiration. The different activities of the spiracles in the anterior part of the body and in the abdomen of adult moths reflect the different mechanisms for tracheal ventilation in these regions. It is remarkable that the spiracles of the first abdominal segment in some non-lepidopterous insects behave like the thoracic spiracles. This implies that in these species the first abdominal segment may belong to the anterior part of the body with respect to its pressure mechanics, as has been postulated on account of the position of the haemolymph pressure valve and the transverse septum in Lepidoptera (Wasserthal 1980).

Comparison of Discontinuous Gas Exchange in Pupae and Adult Insects

Discontinuous ventilation or CO_2 discharge has been reported in representatives of several insect orders (review Miller 1974). It is probable that the discontinuous CO_2 discharge in pharate *P. machaon* and hibernating adult *Vanessa urticae* (Punt 1950) is due to ventilatory movements similar to those described in adult *P. machaon* and *A. atlas*. The relatively high basal CO_2 level between the CO_2 bursts in adult Lepidoptera can be correlated with the slow expiratory phase in the anterior part of the body alternating with expiration in the abdomen.

The cyclic CO_2 bursts in diapausing saturniid pupae, however, are not coordinated with active or passive abdominal movements (Brockway and Schneiderman 1967). It has been assumed that the discontinuous discharge of CO_2 reduces water loss by the maintenance of a negative pressure in the tracheal system. The origin of this negative pressure is ascribed mainly to O_2 consumption during constriction of the spiracular valves for long periods (Buck 1958; Levi and Schneiderman 1958, 1966). A similar mechanism with stationary phases of subatmospheric intratracheal pressure in adult *Periplaneta americana* is also suggested to be advantageous for water retention (Kestler 1980). In *Periplaneta* a period of abdominal ventilatory movements is followed by a period of spiracular valve constriction and a period of spiracular valve 'fluttering'. In the adult *A. atlas* only periods of opened or 'fluttering' spiracles alternate with periods of abdominal ventilatory movements. No corresponding constriction period of the spiracular valves could be observed under relatively optimal experimental conditions, especially a high relative humidity. Since the metathoracic spiracular valves are opened or at best 'fluttering', even during the abdominal ventila-

tory contractions, intratracheal pressure should not exhibit persistent changes but rapidly return to atmospheric pressure. A 'stationary' subatmospheric intratracheal pressure may arise if the pressure compensation through the spiracles is delayed by frequent valve 'fluttering', while a negative pressure is generated in the anterior part of the body by removal of haemolymph and in the abdomen by abdominal expansion.

Discontinuous ventilation in adult saturniids arises in a different way than in their pupae. Apart from the abdominal ventilatory movements, it is a consequence of coupling tracheal ventilation to periodic haemolymph movements between the anterior and posterior parts of the body. One fundamental difference between inactive insect pupae and flight-adapted, light-weight adults concerns the haemolymph volume. Thus adult blowflies possess about 3 to 6 times less haemolymph than their pupae (calculated from data in Jones 1977), and in the sphingid *Celerio euphorbiae* the percentage of haemolymph in body weight is diminished from 18.3–42.6% in the pupa to 2.8–11.6% in the adult (Heller and Sweichowska 1948). It is proposed that the oscillating haemolymph supply in Lepidoptera and Diptera is an adaptation which serves to compensate for a reduced haemolymph volume in the adult stage. Due to the compartmentation of the body into anterior and posterior parts by the transverse septum and the haemolymph pressure valve, haemolymph can be most effectively utilized – even if its quantity is small – as a hydraulic fluid for tracheal ventilation when it is moved back and forth from one compartment to the other. In the saturniid moth this mechanism reveals a high degree of perfection: when removal of haemolymph causes the tracheae in the anterior part of the body to inspire, haemolymph accumulation in the abdomen supports tracheal expiration at the posterior part of the body by virtue of its coincidence with ventilatory contractions.

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