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 CO₂ 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 Philippines 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 (d 0.1 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 (d 0.3 to 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 ΔT 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 haemocoel 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 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 *Bombbyx* (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. 3b) (Richards 1963). In *A. atlas* the anterior abdominal section of the median connective tissue component (VD) of the ventral diaphragm is enlarged (Fig. 3b).