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Antagonism Between Haemolymph Transport and Tracheal Ventilation in an Insect Wing (*Attacus atlas* L.)

A Disproof of the Generalized Model of Insect Wing Circulation

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Summary. 1. The interrelations between haemolymph transport and tracheal ventilation in the wings have been analyzed in resting giant silk moths, *Attacus atlas* L., with respect to the oscillating haemolymph supply of the body.

2. Direction and relative intensity of haemolymph flows in the wing veins are recorded simultaneously with pulse activity of the mesotergal pulsatile organ (PO) by means of contact thermography.

3. The condition of tracheal distension and the haemolymph content in the wing veins are examined by light microscopy and scanning electron microscopy after rapid freeze fixation at specified times during the pulse periods and the pauses of the PO.

4. Haemolymph is periodically sucked out from all wing veins by the PO mainly during reverse pulse periods of the heart and is transported into the abdomen. Haemolymph returns into all wing veins after the first 10 to 25 pulses of the forward pulse period of the heart throughout the pulsation pause of the PO.

5. By removing haemolymph from the wing veins, the POs induce a compensatory increase of tracheal volume and thus bring about wing inspiration. Owing to their elasticity the wing tracheae act as antagonists to the POs, sucking haemolymph back into the wing haemocoel in the course of expiration.

6. Cross-sectional tracheal elasticity is based on coiling of the taenidia along the *transverse* axis of the wing tracheae which is superimposed on the normal longitudinal helical arrangement.

7. The functional advantage of haemolymph oscillation and the possible distribution of this supply mechanism in insect wings are discussed.

Introduction

Insect wings are living evaginations of the thorax wall. In Lepidoptera mechanoreceptors, scent organs and chordotonal organs have evolved along the veins and margins (Freiling 1909; Vogel 1912). The wing veins are equipped with haemolymph sinuses, tracheae and nerves (cf. Fig. 3) and it is generally assumed that wings are supplied with haemolymph and respiratory air. Without haemolymph supply, wings become dry and brittle (Arnold 1964). Haemolymph in the wings has repeatedly been observed to circulate (literature quoted in Verloren 1847; Yeager and Hendrickson 1934; Arnold 1964) and the circulation model has been generally accepted (Weber and Weidner 1974; Wigglesworth 1972; Jones 1977 and most other textbooks). In contrast to the numerous observations of blood circulation almost nothing has been published about respiratory gas exchange in the wing, probably because this is not accessible to direct observation.

It has been suggested that circulation in the wing veins is sustained by the heart which pumps haemolymph into the anterior veins (C, Sc, R, M), and by the thoracic pulsatile organs which suck it back from the posterior veins (Cu, A) into the thorax (Arnold 1964). In resting Lepidoptera the pulse activity of the meso- and metathoracic pulsatile organs is intermittent, their activity periods being coordinated mainly with the reverse pulse periods of the heart and their pauses coinciding mainly with the forward pulse periods (Wasserthal 1976). Haemolymph flow in the wings was postulated to change direction in coordination with haemolymph oscillation in the body (Wasserthal 1976, 1980).

In the present study the haemolymph flow in the wing veins is analyzed in parallel with the pulse activity of the mesotergal organ by means of contact thermography. The interrelation between tracheal ventilation and haemolymph oscillation in the wing veins

Abbreviations: PO mesotergal pulsatile organ; C-method conduction-convection measurement; T-method temperature measurement; wing vein: A analis; C costa; Cu cubitus; M media; R radius; Sc subcosta

is examined after rapid freeze fixation of resting moths at specific times during the PO activity sequence.

Materials and Methods

Saturniid moths, *Attacus atlas*, were chosen because of their large wings. The origin, breeding methods and experimental conditions were similar to those described in Wasserthal (1981). The specimens were resting at their cocoons in a transparent animal cage (20 l) under controlled environmental conditions. The specimens were adapted to ambient temperatures between 21–23 °C at 90% R.H.. Since ambient temperature was regulated at a very low frequency (1 cycle per h, or less) and changed maximally 0.02 °C within 10 min, no ambient temperature reference has been included in the figures.

During temperature measurements of this kind an adequate consideration of the ambient temperature reference in the curves is complicated because the heat storing capacity of the different measuring sites is not identical. Wing parts with a small mass adjust more quickly to changes of T_a than does the thorax with its greater mass: e.g. in Fig. 5 a slight overall temperature increase is apparent at the veins but the temperature course of the body still shows a negative trend. In addition, the thermal characteristics of the different parts of the specimens are periodically changing due to the varying haemolymph content.

Microthermistors were fixed onto the cuticle (after removal of the scales) of one or two veins (6–8 or 12–16 mm distant from the base) of the anterior wings, and simultaneously on the mesoscutellum for measurement of the activity of the accessory pulsatile organ. The natural temperature fluctuations caused by the slight metabolic warming of the resting moths were recorded with practically unheated thermistors ($\Delta 0.35 \pm 0.05$ °C, T-method, Wasserthal 1980).

To visualize the pulses of the mesotergal pulsatile organ and the haemolymph flow in the wing veins the convective and conductive cooling effects exerted on a heated thermistor ($\Delta 1.7$ – 1.8 °C) by haemolymph movements and by changes in haemolymph and air content just below the measuring site, were measured with the same thermistor (C-method, Wasserthal 1980).

The direction of haemolymph flow in the veins was analyzed by temperature measurements with the T-method by *locally* heating the haemolymph in the wing vein by means of a second thermistor at sites distal or basal to the measuring site. This is indicated in the figures. While the measuring microthermistor bead (Veco 32a 402a) was 0.1 mm in diameter, the heat-marking thermistor had a larger diameter of 0.25 mm (ITT U 23S). At the costal vein of the anterior wing, the thermistor heating effect was insufficient so that heating was performed with a fibre glass lamp (Schott KL 150) which was used to irradiate a 5 mm circular wing area with the ITT thermistor at its centre to control the excess wing temperature ($\Delta 0.7 \pm 0.1$ °C) caused by the lamp. The local heat-marking of the wing haemolymph did not significantly influence the T- and C-curves of the PO.

Changes in tracheal and haemolymph volume in the wing veins parallel to the intermittent activity of the pulsatile organ were examined after rapid freeze fixation of whole animals in 2-methyl butane cooled to -140 °C with liquid N_2 . The cocoons with the resting specimens were suspended by a thin thread above the vessel containing the cooling fluid. After recording the PO periodicity with the C-method for 2 h without disturbance, the suspending thread was cut at an exactly defined moment during the PO activity sequence. The wing veins were then fractured in the freezing fluid at the level of the outer red-white band of the wing pattern (Fig. 9) and transferred to a freeze drying apparatus. Sublimation needed 2–3 d at 0.03 mbar. After identification of the dried wing fragments under N_2 gas atmosphere, vein fragments

from the level of the red-white band were embedded in Epon. Semi-thin sections were analyzed by phase contrast microscopy. The cross fractures of all other fragments were examined under the stereomicroscope. The intima surface inside the freeze-fractured veins was analyzed by aid of a SEM (ISI S III A) after sputtering with gold. Only those specimens which did not visibly react during freezing were used, i.e. if the antennae were preserved in unchanged resting position and, in the case of females, if the genitalia were extended in alluring position ($n=26$).

Results

Intermittent Transport Activity of the Accessory Tergal Organs

The meso- and metatergal pulsatile organs in the giant silk moth exhibit intermittent pulse activity with pulse periods and pauses which are coordinated with the heartbeat sequence (=forward pulse period + pause + backward pulse period) (Wasserthal 1976). The pulse activity of both tergal organs always begins with some delay after the onset of reverse heart beating and ends within the first minute of the forward pulse period of the heart.

The temperature curves (T-method) of the mesoscutellum above the mesotergal organ (PO) show weak PO pulses and a slight warming during the course of PO pulse activity with a maximum toward the end of the PO pulse period (Fig. 1). Since this warming accompanies the PO pulse activity and is correlated with a slight increase of pulse amplitude, it is assumed that it results mainly from the metabolic warming of the PO muscles.

The C-curves from the mesoscutellum look fundamentally different (Fig. 2a). Their marked changes in temperature level reflect two superimposed phenomena: 1) Variation in thermal conduction as a consequence of periodic change in haemolymph volume in the anterior body caused by haemolymph oscillation and 2) variation in thermal convection caused by discontinuous haemolymph transport mainly of the PO.

The comparison of the T-curve with the C-curve from the mesoscutellum shows that the small natural temperature fluctuations of less than $\Delta 0.02$ °C are negligible with regard to the estimation of the conductive and convective effects of the C-curve (Figs. 1, 2a, 2d). On the other hand, the distinct convective and conductive cooling effects, which are visualized by the heated thermistors in the C-curve, are not superimposed on the temperature fluctuations of the T-curve. This shows that the applied T-method is not merely an insensitive C-method, and that the slight thermistor excess temperature of $\Delta 0.35$ °C has no unwanted influence upon the measurement of the natural temperature fluctuations as performed in this study. See also page 32.

The contribution by *conductive* cooling can be assessed on the basis of the C-curves from the head in *A. atlas* and the mesoscutellum in *Papilio machaon*, where no PO activity interferes (Wasserthal 1980,

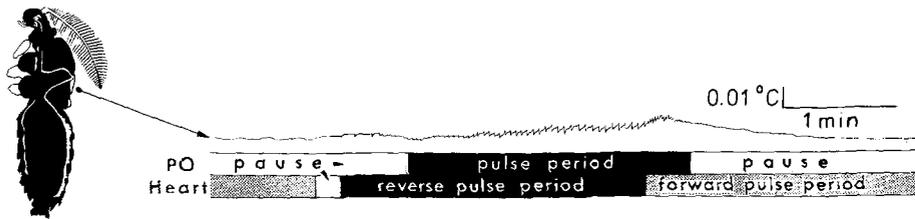


Fig. 1. Natural temperature fluctuations at the mesoscutellum above the mesotergal pulsatile organ (PO) of a resting moth adapted to a constant ambient temperature of 22 °C. The slight warming in the course of the PO pulse period is probably caused by the muscle activity of the PO (T-method)

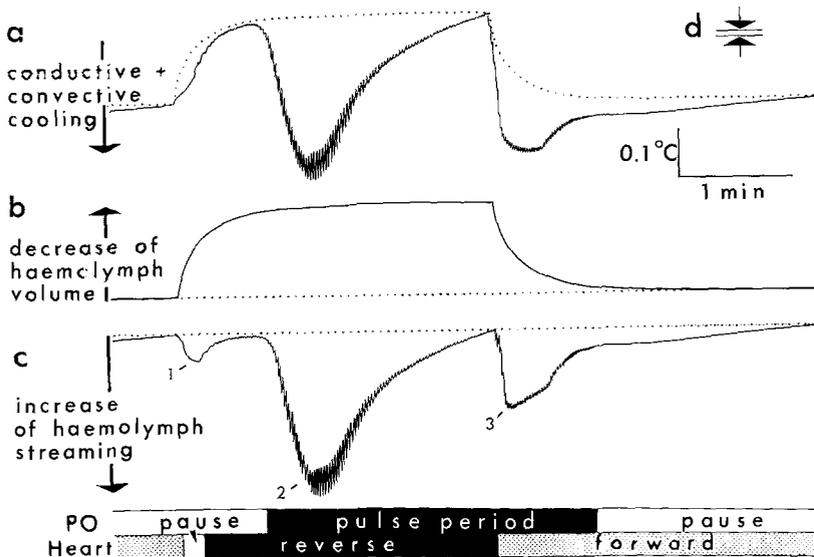


Fig. 2. a Intermittent pulse activity of the mesotergal organ and changing haemolymph content and flow rate below the mesoscutellum visualized by heating the measuring thermistor to a Δ of 1.8 °C (C-method). Same specimen, thermistor site and conditions as in Fig. 1. b, c Schematic explanation of the temperature changes of the C-curve: b Relative contribution of conductive cooling exerted on the heated thermistor site by changing haemolymph content of the thorax: The decrease of haemolymph volume in the thorax during pause and reverse pulse period of the heart reduces conductive cooling. c Relative contribution of superimposed convective cooling. Numbers 1, 2, 3 indicate events of increased flow rate. d Total range of natural temperature fluctuations from the T-curve in Fig. 1 at same scale

1981). This is shown schematically in Fig. 2b. The contribution by *convective* cooling (curve c in Fig. 2) is obtained by subtraction of curve a from curve b. This procedure reveals three different phasic convective cooling events in the course of one heartbeat sequence, each of which corresponds to an increase of the mean flow rate. The first, rather weak convective cooling event (numbered 1 in Fig. 2c) occurs during pausing of heart and POs. This flow increase is attributed to the sucking effect of the expanding abdomen (Wasserthal 1981). The other two convective cooling events follow in the course of the PO pulse period: The highest mean transport rate is achieved during *reverse* beating of the heart (numbered 2 in Fig. 2c). This event is accompanied by an increase of pulse amplitude and reflects the main PO transport phase, during which most of the wing haemolymph is sucked by the PO into the thorax (see below) and is pumped via the aorta and heart into the abdomen. The third phase of convective cooling coincides with haemolymph return through the aorta at the onset of the *forward* pulse period of the heart (no. 3 in Fig. 2c): Since only a small quantity of haemolymph is left in the wing veins and in the thorax from the end of the backward pulse period

until the about 10th first forward pulse of the heart (see p. 33), the abrupt beginning of this third convective cooling event is probably caused mainly by the forward pulses of the aorta, which lies close to the mesotergal PO, and to a smaller extent directly by the pulses of the mesotergal PO. At the *metatergal* PO, which is more distant from the aorta, the corresponding event of convective cooling sets on less abruptly (see Fig. 2b in Wasserthal 1976). As soon as the haemolymph returns into the lateral meso- and metathoracic haemocoelae, both POs stop beating. They do not renew their pulse activity before the haemolymph content of the thorax has been reduced again by abdominal suction and by the following backward pulses of the heart (Wasserthal 1981).

Morphology of the Wing Veins and Wing Membrane

The wing veins are composed of rigid sclerotized cuticular channels of the lower wing lamina and an almost flat covering of the upper wing lamina (see below, Figs. 10, 11). The channels are U-shaped in cross section and possess cuticular ledges for support of the rooflike upper lamina. Only the costal vein of the forewing has a different, asymmetric shape and does

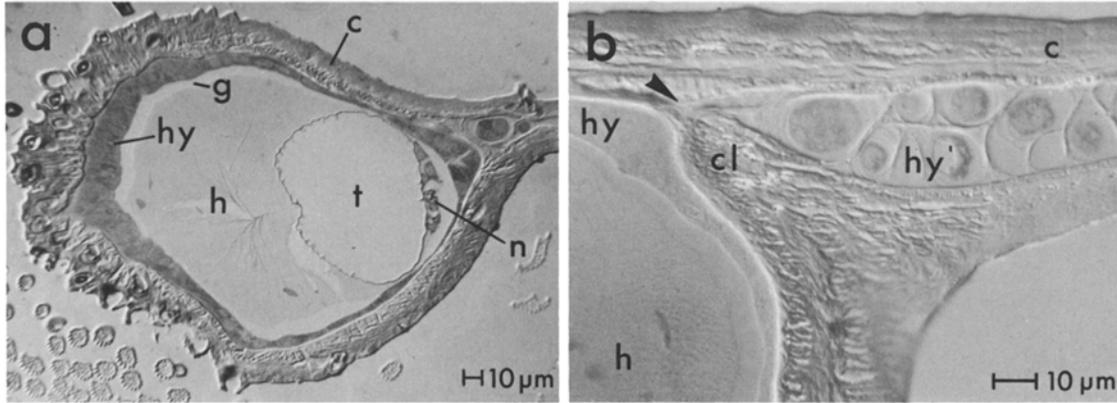


Fig. 3a, b. Morphology and preservation of a freeze-fixed cross-sectioned wing; interference contrast. **a** Costal vein. The haemocoel of the vein is completely lined by the hypodermal cell layer (*hy*). *g* artificial gap caused by shrinking of the haemolymph substance (*h*) after uptake of atmospheric humidity before embedding in Epon. The dendritic structure in the haemolymph is caused by decomposition and formation of ice crystals during freezing. *c* thermistor site on the vein cuticle; *n* nerve; *t* tracheal lumen. **b** Transition between (subcostal) wing vein and wing membrane; orientation according to frame in Fig. 10a. No haemocoel lacunae occur between the hypodermal cells of the wing membrane (*hy'*). There is only a narrow connection (*arrowhead*) from the hypodermal cells of the vein (*hy*) to the wing membrane between upper cuticular lamina (*c*) and cuticular ledge (*cl*) of the lower lamina

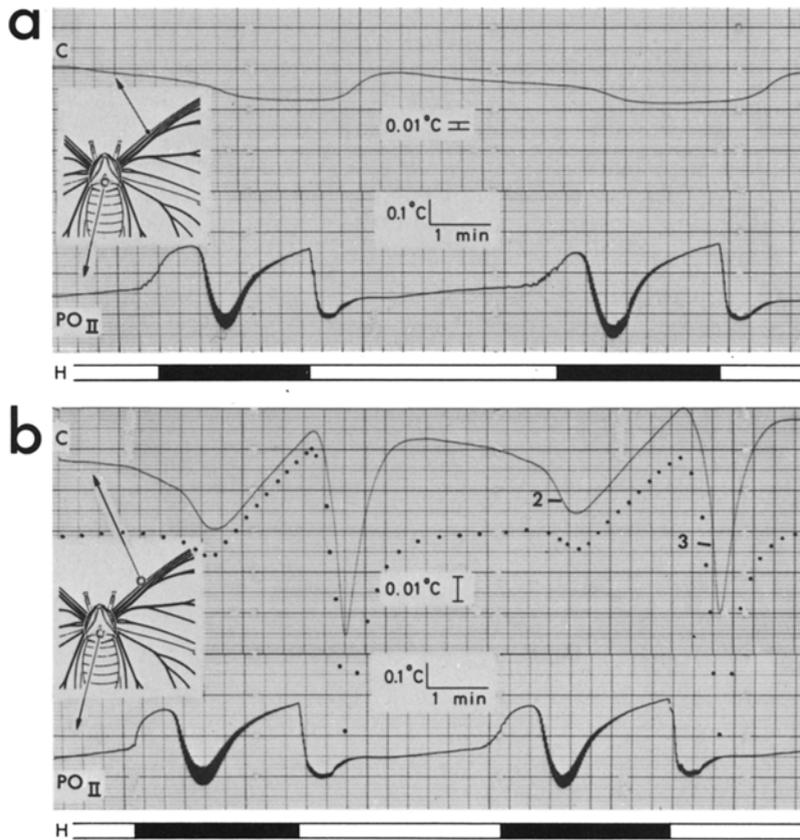


Fig. 4a, b. Influence of intermittent activity of the mesotergal pulsatile organ (*PO_{II}*) (C-method) upon natural haemolymph temperature fluctuations and flow in the costal vein (C). 2 day old female.

a T-method: Inflow of body haemolymph (carrying metabolic heat) into the vein during PO pause.

b C-method: Two phases of intensified haemolymph flow (= convective cooling) during one PO activity sequence; one phase (no. 2) during backward pulse period, another phase (no. 3) during forward pulse period of the heart. *Black bar* defines backward pulse period, *dotted line* C-curve corrected for natural temperature fluctuations and slight increase of ambient temperature

not have the cuticular ledges (Fig. 3a). Between the stabilizing ledges and the cuticular roof only a narrow cleft for cell contacts from vein toward membrane is left (Fig. 3b). The inner surface of the vein cuticle is completely lined by a hypodermal cell layer which also separates the vein haemocoel from the wing

membrane. No haemocoel lacunae could be found between the two hypodermal cell layers of the wing membrane. The central lumen of the veins is occupied by a single large trachea accompanied by a nerve, both surrounded by haemolymph. Cross veins are absent in the wings of *A. atlas*.

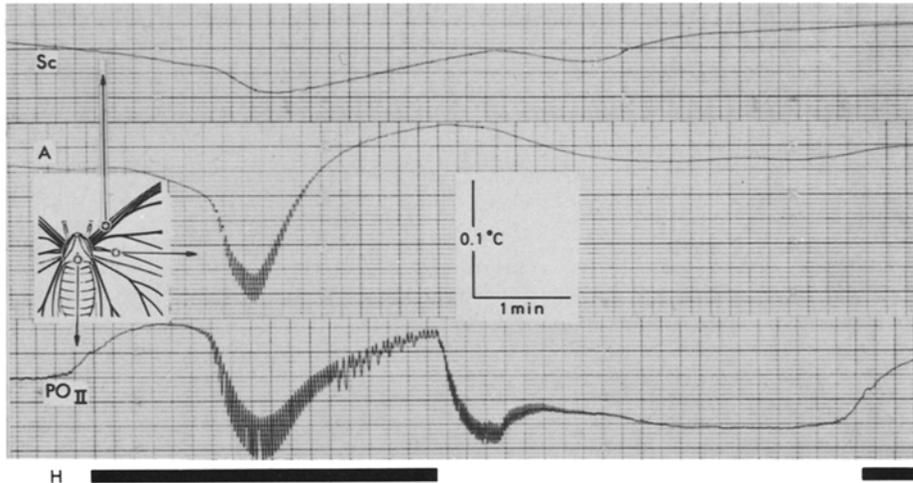


Fig. 5. Temporal coincidence of flow events in an anterior (Sc) and a posterior vein (A) in relation to PO periodicity. 2 day old female (C-method). Black bars define reverse pulse period of the heart

Temperature Measurements (T-Method) Without Heat-Marking of Wing Haemolymph

At the wing veins slight variations of the temperature level can be recorded periodically (Fig. 4a). At the costal vein (C) these changes are distinct with an amplitude of $\Delta 0.03^\circ\text{C}$. At the subcostal vein (Sc) this amplitude is between $\Delta 0.03\text{--}0.01^\circ\text{C}$. At all other veins the difference between the highest and lowest T-level is less than 0.01°C and therefore almost undetectable. At all veins these periodic temperature variations occur in correlation with the periodicity of the PO: At the beginning of the inactivity period of the PO, the temperature in the veins rises. This T-rise lasts maximally for only 1 min. Then a slow cooling phase follows which terminates at the end of the PO pulse period, when the next warming phase begins. The cooling rate is most intensive at the time when the convective cooling of the PO pulses at the mesoscutellum is most effective, i.e. during the first half of the reverse pulse period of the heart.

Since – due to metabolic heat – the body temperature of resting *A. atlas* is slightly above ambient (Wasserthal 1981), the temperature rise in the wing veins at the end of the PO pulse period suggests an equidirectional inflow of haemolymph from the body into all veins.

Periodic Variations in Haemolymph Flow Intensity in the Wing Veins (C-Method)

After heating the measuring thermistor to about $\Delta 1.8^\circ\text{C}$ above ambient, haemolymph flow in the veins could be detected by its convective and superimposed conductive cooling effect upon the thermistor site. The C-curves of all wing veins are characterized by two cooling phases during the course of each heart-

beat sequence, one during the backward pulse period, the other during the forward pulse period. They represent two different flow events (Figs. 4b, 5, 6):

1. During the backward pulse period of the heart, haemolymph flow in the veins coincides with the increase of the PO transport rate. Only in the posterior vein, however, is the highest volume flow rate (moment of lowest temperature) synchronous with the highest transport rate of the PO (Fig. 5: A). In all other (more anterior) wing veins it is observed with some delay respective to the peak transport rate of the PO (Figs. 5, 6). At the more posterior veins (R, Cu, A; Figs. 5, 6), the single PO pulses are distinct, whereas they are scarcely perceptible at the anterior veins (C, Sc; Figs. 4b, 5).

2. The other flow event in the wing veins during the forward pulse period of the heart does not coincide with the return of haemolymph into the aorta (convective cooling event no. 3 in Fig. 2c) but begins later when the PO pulse activity is ceasing. At the anterior (costal) vein this flow event becomes visible first. It begins rather abruptly and lasts for only a short time in the more anterior veins (C, Sc, R; Figs. 4b, 5, 6a). This is in contrast to the posterior veins (Cu, A; Figs. 5, 6b) where it begins more gradually and lasts until the end of the PO pause.

The natural temperature fluctuations of the costal vein and, to a minor extent, of the subcostal vein are significant and so must influence the C-curves. Therefore it was attempted to calculate a C-curve in which these natural temperature fluctuations are eliminated (Fig. 4b: dotted line). It is obvious that the convective cooling event during the backward heart pulses (numbered 2) almost disappears whereas the convective cooling event during the forward pulse period of the heart (numbered 3) is amplified. It must,

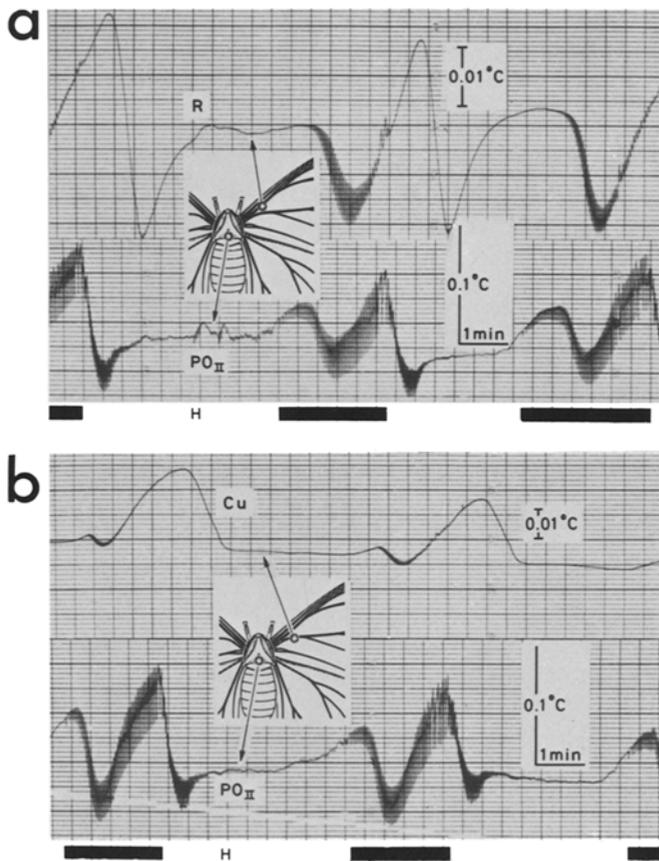


Fig. 6a, b. Temporal coincidence of haemolymph flow in the radial (R) and the cubital (Cu) veins during the PO pulse period and at the beginning of PO pause. **a** 3 day old female; **b** 4 day old female (C-method). While in R the cooling at the onset of the PO pause lasts only for about 30 s it continues throughout the entire PO pause in Cu. Black bar shows reverse pulse period of the heart

however, be considered that the natural temperature fluctuations (Fig. 4a) may already comprise some natural convective cooling: During the centripetal haemolymph flow, the cooler peripheral haemolymph might convect heat from the basal vein cuticle, which had been stored there during the preceding centrifugal flow period. In the calculated C-curve, the convective cooling effect (no. 2) which is attributed to the centripetal flow, is therefore probably underestimated.

An additional superposition of a convective and conductive cooling effect in the costal T-curve might theoretically be attributed to the $\Delta 0.35^\circ\text{C}$ excess warming of the thermistor. Such an influence is, however, improbable, because the total amplitude of the costal C-curve is only about 1/3 that of the C-curve from the PO (Fig. 2) which had no visible influence upon the course of the corresponding T-curve (Fig. 1).

After the first flow event during the reverse pulse period of the heart, the temperature reaches a higher level than after the second flow event in the course of the forward pulse period at all wing veins. These differences in temperature level are relatively small

in the 'uncorrected' C-curve of the costal and subcostal vein (Figs. 4, 5), but they become conspicuous in the 'corrected' curve of the costal vein (Fig. 4b) and the curves of the radial (R), cubital (Cu) and anal (A) veins (Figs. 5, 6).

Conclusions. The different temperature level after both convective cooling phases suggests that both flow events are accompanied by a change in thermal conduction, probably due to a different haemolymph volume in the wing veins. Thermal conduction, or haemolymph volume, of the veins are assumed to be higher at the end of the PO pause and lowest shortly before the end of the PO pulse period. From the differences of the flow intensity and the clearly visible PO pulse effects at the different veins it can be concluded that the haemolymph flow during the reverse pulse period is more vigorous at the posterior veins (Cu, A), whereas the flow event during the forward pulse period is more vigorous, but also shorter at the anterior veins (C, Sc). In the intermediate radial vein both flow events are likewise vigorous. The sucking force of the expanding abdomen (flow event no. 1 in the C-curve of the PO, Fig. 2c) and the abdominal ventilatory movements, which occur during the reverse pulse periods of the heart, have no significant effects in the C-curves of the veins.

Periodic Change in Flow Direction of Wing Haemolymph (T-Method with Heat-Marking)

Even though the appearance in the wing veins, of warmer haemolymph from the body (as measured with the T-method alone; Fig. 4a) indicates a periodic haemolymph inflow, the flow direction could be determined unambiguously only by combining the temperature measurement with local heat-marking of wing haemolymph, either distally or proximally from the measuring thermistor. Heating of wing haemolymph *distally* from the measuring thermistor revealed that within all veins warm haemolymph streams *toward the body* during the phase of intensified haemolymph transport of the PO (no. 2 in Fig. 2c), i.e. when the heart is beating backward (Figs. 7a, 8). During the following flow phase in the wings, parallel with the forward pulse period of the heart, haemolymph from the body flows past the measuring thermistor in distal direction through the veins. This is confirmed by a control experiment in which wing haemolymph is heated proximally from the measuring thermistor (Fig. 7b). The resulting curves demonstrate the same equidirectional change of haemolymph flow throughout all wing veins: The cooling phase during the main PO transport phase indicates that unheated haemolymph flows toward the wing bases. The wing warm-

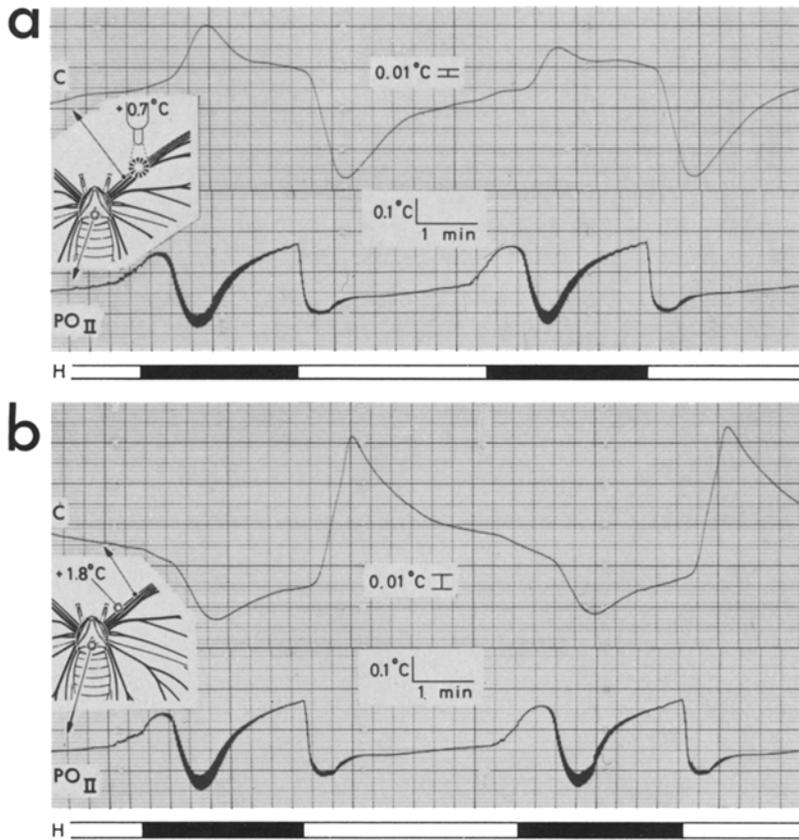


Fig. 7a, b. Periodic reversal of haemolymph flow in the costal vein during one PO activity sequence. 2 day old female.
a T-method with heat-marking of haemolymph apically from the measuring vein thermistor: The warming at the vein during the PO pulse period indicates that haemolymph flows *centripetally*.
b T-method with heat-marking of haemolymph basally from the measuring vein thermistor: Warm haemolymph passes below the measuring vein thermistor at the end of the PO pulse period, indicating a *centrifugal* streaming. Black bar shows reverse pulse period of the heart

ing after termination of the PO pulse period shows that haemolymph from the heated wing base flows toward the wing apex. The curves obtained after basal heat-marking thus run inversely to the curves recorded under apical heat-marking. Heat-marking of haemolymph basally from the measuring thermistor amplifies the effects observed in the T-curves (without artificial heat-marking) by adding thermistor heat to the metabolic heat (Fig. 4a).

Tracheal and Haemolymph Volume of the Wing Veins at Specific Times During the PO Activity Sequence

To test the postulated interrelation of tracheal and haemolymph volume in the wing veins, moths were freeze-fixed at different phases of the PO activity sequence. The tracheal condition and degree of haemolymph content could already be recognized under the stereomicroscope in all cross fractures of the veins from the base to the outer margin of the freeze-dried wings: The relation of the white haemolymph material and the dark lumen of the tracheae was similar in different regions along the same vein as well as in different veins of fore- and hindwing. For better comparison histological sections are shown at the level of half the wing length, approximately along the

red-white band of the wing pattern (Fig. 9, arrows, Figs. 10 and 11).

When fixed shortly before or at the end of the PO pause ($n=6$), the wing veins contain the maximal amount of haemolymph and their tracheae have a relatively small and circular lumen in cross section (Figs. 10a, 11a). Curiously, only the anal veins of the anterior and posterior wings sometimes (3 specimens) exhibit compressed tracheae (Fig. 11a: A), but these look as if they had been artificially deformed, possibly by formation of large ice crystals due to less rapid freezing.

The lumen of the wing veins from moths which had been fixed shortly before the end of the PO pulse period ($n=6$) is almost entirely occupied by the tracheae, with only a small haemolymph quantity filling the space in the corners adjacent to the wing membrane (Fig. 11b). The tracheal wall and haemolymph are sometimes detached from the vein wall by shrinkage of the haemolymph material (Figs. 3a, 10b: A).

In all wing veins, fixation during a longer timespan – between the end of the reverse pulse period and the end of the PO pulse period – results in clearly expanded tracheae. In the costal vein of the anterior wing, however, the extremely expanded condition of the trachea is obtained only during the short phase

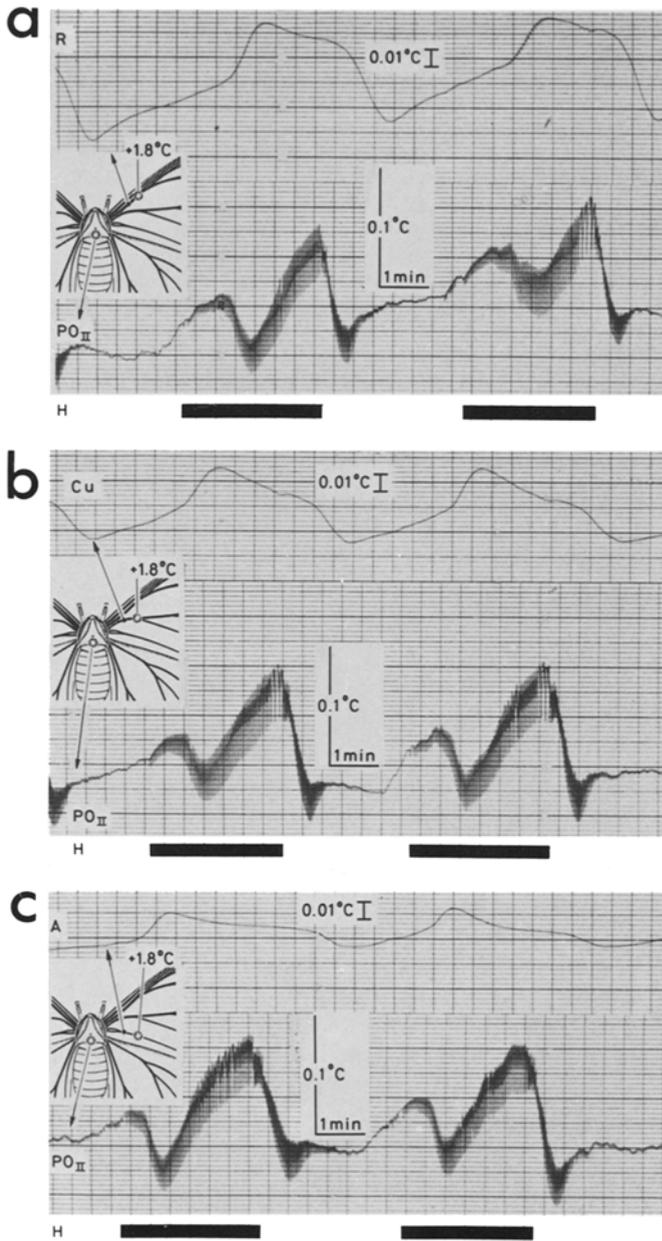


Fig. 8a-c. Equidirectional haemolymph flow reversal in Radius (*R*), Cubitus (*Cu*) and Analis (*A*). **a, b** 3 day old female, **c** 4 day old female. T-method with heat-marking apically from the measuring thermistor. The warmed centripetal haemolymph flow passes below the measuring vein thermistor during the reverse pulse period of the heart. The recooling centrifugal flow passes at the wing basis during the forward pulse period. *Black bar* indicates reverse pulse period of the heart

between the end of the reverse pulse period and about the first 10 forward pulses of the heart. If the specimens are fixed exactly at the end of the PO pulse period, the haemolymph content of the Costa is already increased and the tracheal lumen is small (Fig. 10b: C). In this vein relatively more haemolymph surrounds the trachea even during the phase

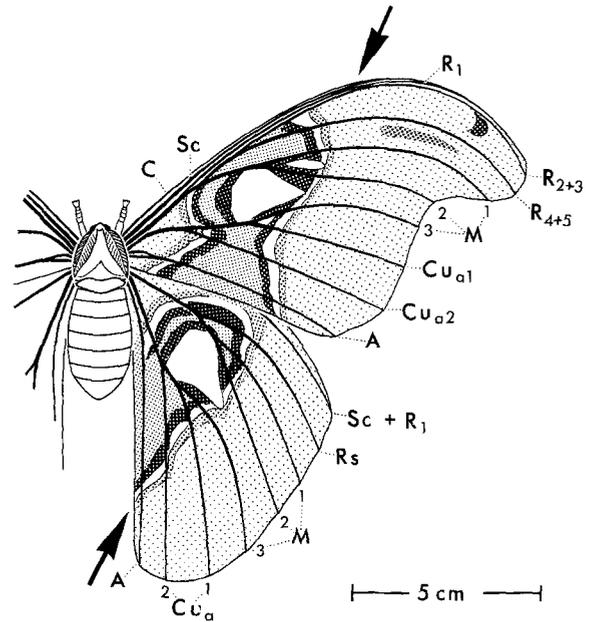


Fig. 9. Wing venation in *Attacus atlas* and level of sections (arrows) in Figs. 3, 10, 11. Note the lack of cross veins

when the latter is expanded maximally (Fig. 11b: C). A comparison of 2 and 6 day old specimens which have been freeze-fixed at identical times during the PO activity sequence, shows that within this lifespan the relations of haemolymph and tracheal volume in the wing veins do not change visibly.

Ultrastructure of the Intima Surface of Wing Tracheae

For analysis of the observed changes in tracheal diameter the intima surfaces of the freeze-fixed vein fragments were studied in the scanning electron microscope. The intima of the wing tracheae is characterized by a coiling of the taenidium along the transverse tracheal axis which is superimposed on the well-known helical arrangement of the taenidium along the longitudinal axis of the tracheae (Fig. 12). The degree of taenidial coiling is clearly correlated with the extent of tracheal distension. In the contracted state the taenidium shows about 23 windings per 100 μm compared to about 14 windings per 100 μm in the expanded state. A fully straightened condition was never observed. Between neighbouring taenidial spirals the surface of the contracted tracheal cell shows prominent wrinkles which are mainly oriented parallel to the longitudinal axis of the trachea, whereas in the expanded trachea the cell surface looks smoother and the surface structures are more transversely oriented.

Summarizing Conclusions. Haemolymph is periodically sucked from all wing veins into the thorax by

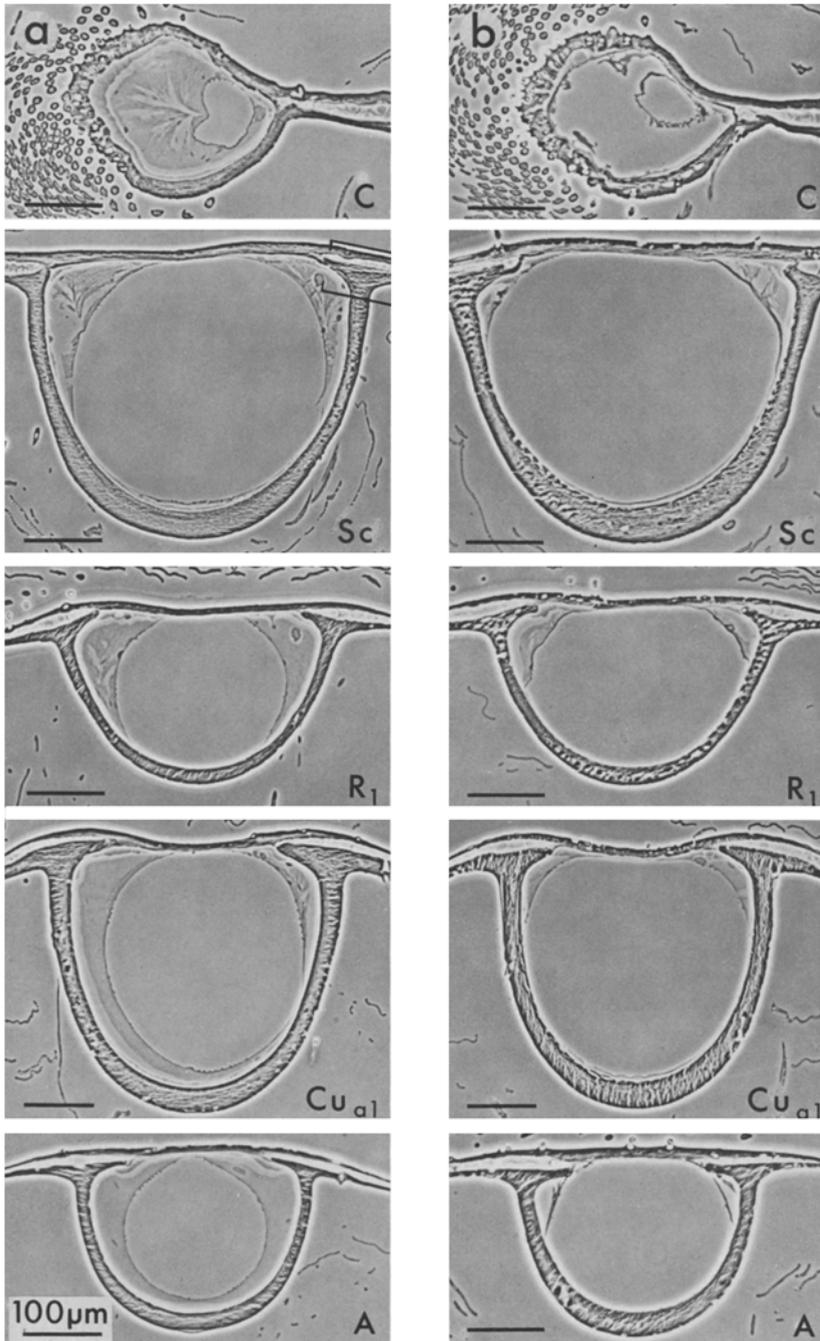


Fig. 10a, b. Changes of haemolymph content and tracheal diameter in the veins of the forewing, related to PO activity in 2 day old specimens. Cross-sections in phase contrast.

a Fixation at the end of PO pause: The tracheae are surrounded by haemolymph and have an almost circular lumen.

b Fixation at the end of PO pulse period: The tracheae fill almost the entire haemocoel of the vein, except for the Costa (C). Framed Sc section (a) shown enlarged in Fig. 3b

the activity of the pulsatile organs, mostly during the backward pulse period of the heart (flow event no. 2 in Figs. 2, 4b). During the forward pulse period of the heart, when the POs stop beating, haemolymph returns from the body into all wing veins (flow event no. 3 in Fig. 4b). Thus, wing haemolymph oscillates in the wing veins, but does not circulate.

A different pathway for the *measured* haemolymph flow, e.g. via cross passage through the wing membrane or along the outer wing sinus can be ruled

out for the following reasons: Haemolymph transport via the wing membrane would be possible only through the intercellular spaces because of the lack of cross veins and lacunae. It would not be very effective. Furthermore, interruption of the encircling wing sinus by damage of the outer wing margin in *Attacus* or by severing the wing membrane from the outer margin towards the wing base (which was performed in *Sphinx ligustri*) did not abolish or reduce the flow effects in the veins (Wasserthal, in preparation).

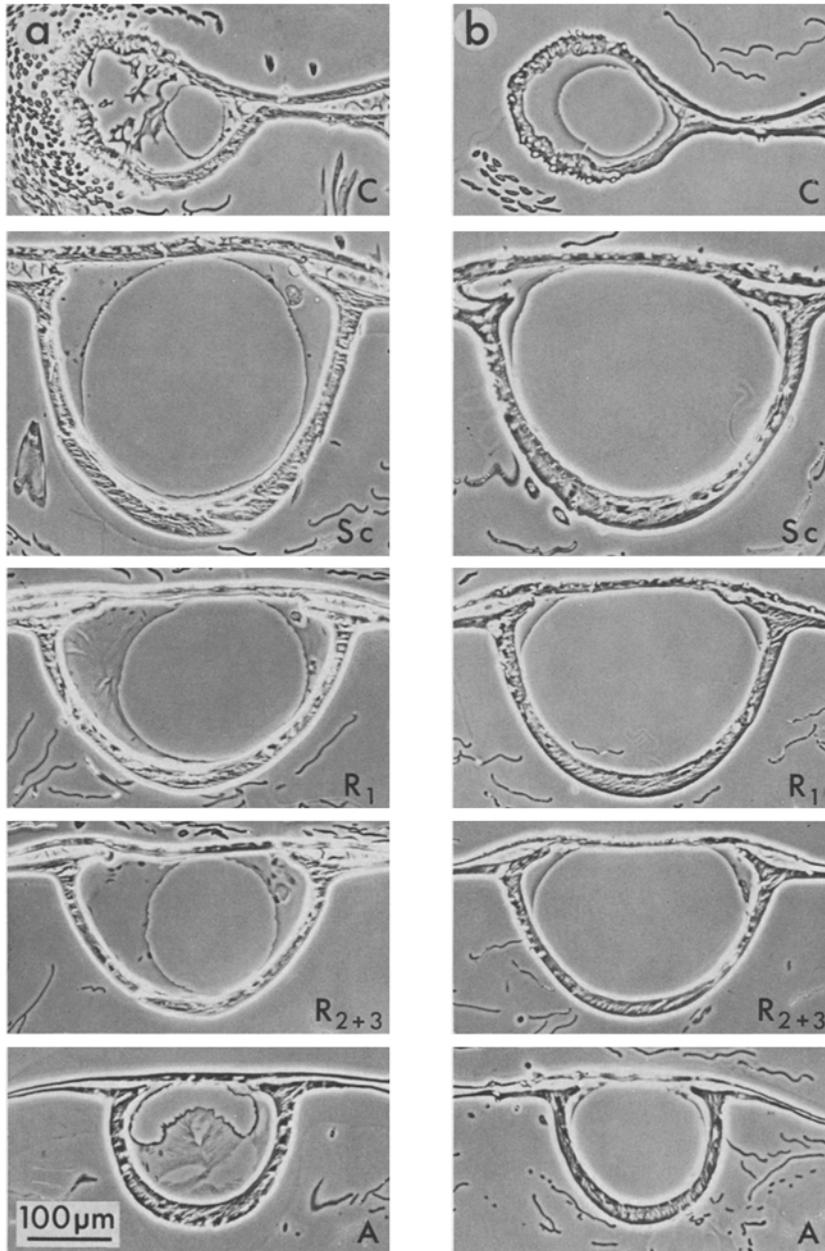


Fig. 11 a, b. Changes of haemolymph content and tracheal diameter in the veins of the forewing, related to the heart activity sequence in 6 day old specimens. Cross-sections in phase contrast, same scale.

a Fixation at the end of the forward pulse period of the heart: The tracheae are surrounded by haemolymph and have a small circular lumen except for the *Analis* (*A*).

b Fixation just after the end of the backward pulse period of the heart: All tracheae show a wide lumen

The periodic changes in haemolymph volume of the wing veins are compensated by changes in tracheal volume. While the haemolymph is being sucked out from the veins, the tracheal walls become maximally extended. This tracheal extension depends – at least partly – on the elasticity of the intima, especially on the stretching of the taenidial coil along the transverse axis.

Apart from a few exceptions, the tracheae of the wings were not found to be compressed during maximum haemolymph filling. This suggests that generally no positive pressure arises in the vein haemocoel. This is consistent with own observations that the elastic pleural membranes in the thorax, which in the young adult

(before the end of post-ecdysial diuresis) are periodically inflated by the positive haemocoelic pressure during forward pulse periods of the heart, later on remain collapsed, thus indicating a persistent negative haemocoelic pressure and a constant outer shape of the thorax in fully developed adults.

The extension of the elastic tracheal walls is maintained until haemolymph returns into the anterior body by renewed forward beating of the heart. When haemolymph is available at the wing bases, the elastic contraction of the wing tracheae sucks it back into the vein haemocoel surrounding the trachea. This filling process is accomplished first and more rapidly in the anterior vein due to its proximity to the anterior

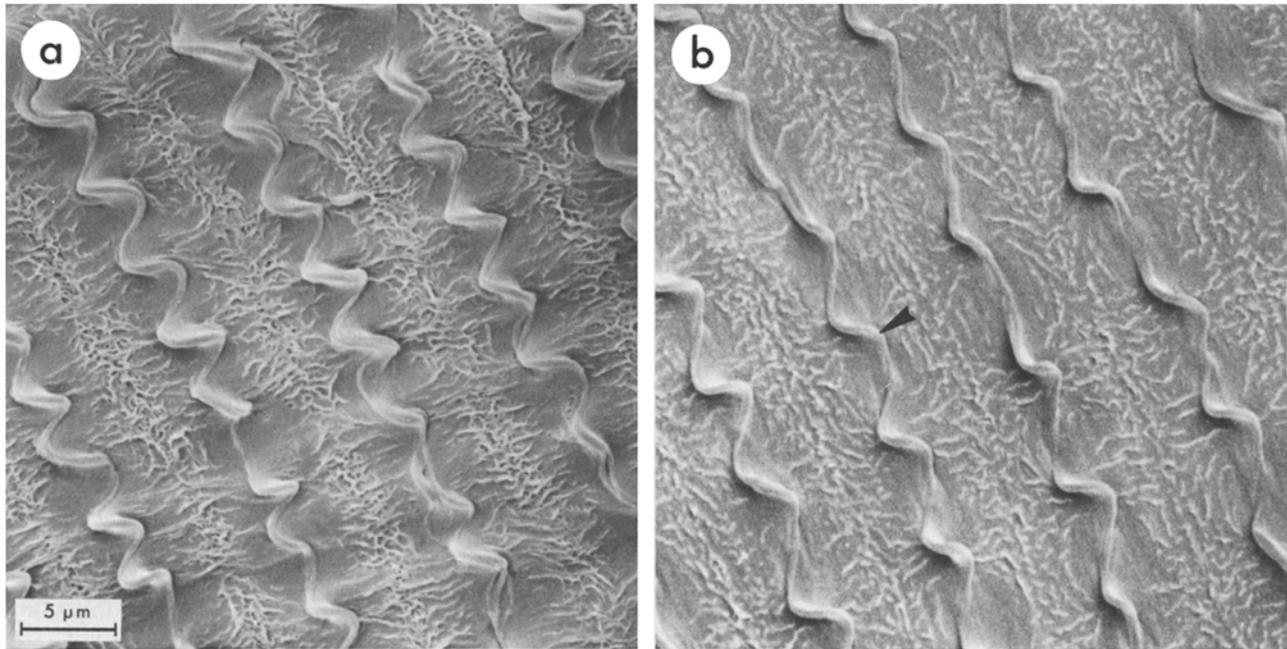


Fig. 12a, b. Intima surface of (subcostal) wing tracheae with taenidial coiling (*arrowhead*) along the transverse axis of the tracheae. Both specimens 2 day old; same scale. **a** Contracted condition (freeze-fixed at the end of PO pause); **b** expanded condition (freeze-fixed at the end of backward pulse period of the heart)

thorax. Here haemolymph appears first after leaving the frontal aorta. The more posterior veins will not receive returning haemolymph before the anterior vein is replenished. The fact that haemolymph can enter the wing veins, especially the costal vein, although the PO is still pulsating, clearly demonstrates that the sucking force of the PO cannot prevent thorax haemolymph from entering the wing veins – especially the anterior ones – as soon as it is available at the wing base, and when the walls of the wing tracheae are under high tension. This is why the PO pulses only during the very first forward pulses of the heart continue to suck haemolymph from the wing veins into the thorax. The following PO pulses must transport some of the returning thorax haemolymph and distribute it in the lateral thorax, thus facilitating an equal supply of the more posterior veins of the forewing and the posterior wings. The way this is brought about is indicated by the different time of entrance of haemolymph into the costa (beginning after about 10–15 forward pulses) and the more posterior veins (beginning after 15–25 forward pulses). Since the force of the POs operates antagonistically to the tension of the wing tracheae, it might prevent a further influx of thoracic haemolymph into the wing veins, if the PO continued to pulsate after the tension of the wing tracheae has declined to a lower, critical level. This, however, is not the case: PO pausing just at this time must be considered to be an essential function which allows the wing tracheae – especially

the posterior ones – to continue relaxation and thereby complete their task of drawing haemolymph back into the wing veins.

Discussion

This investigation has revealed the following mechanism of haemolymph and respiratory gas supply to the wings: By removing haemolymph from the wing veins the POs induce a compensatory increase of tracheal volume and thus act as a motor for active inspiration into all wing tracheae. Owing to their elasticity, the wing tracheae act as a suction device for centrifugal haemolymph filling of the wing sinuses, at the same time causing expiratory air flow. This mechanism explains the origin of the negative pressure in the wing haemocoel, which has been pointed out by Brocher (1931) and Jones (1977), but which has never been understood.

The observed changes in tracheal diameter were unexpected because they cannot be accounted for by the elasticity characteristics of insect tracheal tubes with a smooth annular or helical taenidium – such a pattern is optimally adapted to resist pressure changes. A variety of different intima patterns have been described on the basis of transmission electron microscopy (Richards and Korda 1950). The wing vein tracheae in *Attacus* represent a new morphological type: The taenidial spiralization along the transverse tracheal axis offers the mechanical characteris-

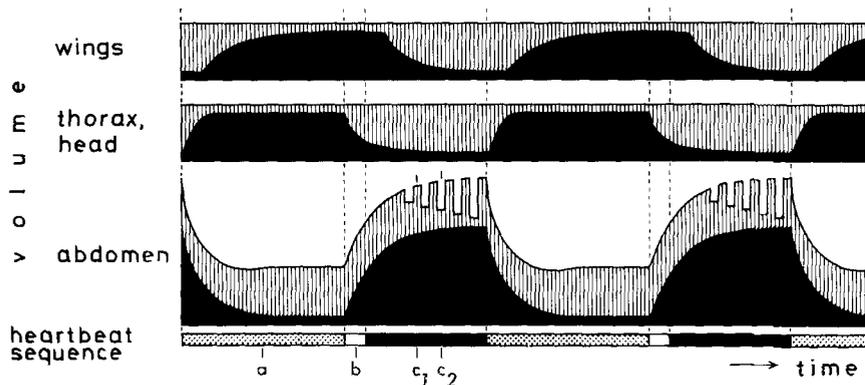


Fig. 13. Interrelationships between tracheal and haemocoel volumes in resting *Attacus atlas*. The total volume of the wings and of the anterior body is assumed to be constant (see text). The total volume of the telescopic abdomen is variable. *Black* haemocoel volume; *vertically shaded* tracheal volume. *a* forward pulse period; *b* pause; *c* backward pulse period of the heart. While in the anterior body and wings the tracheae are slowly ventilated by the oscillation of haemolymph in the course of one heartbeat sequence, the abdominal tracheae are ventilated by bouts of peristaltic movements: c_1 relaxation; c_2 contraction during backward beating of the heart

tics of a traction spring which stores energy in the stretched condition.

The direct demonstration of changes in tracheal lumen and in taenidial coiling caused by oscillation of haemolymph in the wing veins is an important proof of the interdependence of tracheal ventilation and oscillating haemolymph supply in the body of resting adult Lepidoptera (Wasserthal 1981): It confirms the importance of abdominal expansion and subsequent coordinated backward heart beating for production of a negative pressure in the thorax as a prerequisite of haemolymph transport from the wings into the body. The POs can only suck wing haemolymph into the thorax following haemolymph removal from the anterior body into the abdomen. Thus three motors – abdomen, heart and POs, switched on and off metachronously in a sophisticated way like vacuum pumps in series – are engaged in haemolymph and gas supply of the wings. The interplay of haemolymph oscillation, between wings and anterior body and between anterior body and abdomen, with tracheal ventilation is summarized in Figs. 13 and 14.

This oscillation mechanism conflicts with the generally accepted model of wing circulation which comprises haemolymph inflow into the wing via the anterior veins (C, Sc, R, M) under cardiac pressure and outflow via the posterior veins (Cu, A) by the sucking force of the POs (Arnold 1964). Only Zeller (1938) has denied the existence of wing circulation. In *Ephestia* wings, especially of pharate adults, he had observed an alternating centrifugal and centripetal movement of stain particles, obviously caused by single PO pulses. Like other authors he supposed that haemolymph would enter the wings under positive pressure.

An investigation of the numerous observations upon which the circulation model was based reveals contradicting facts which, however, can be interpreted as events in an oscillating wing supply: Haemolymph does not circulate continuously but after a pause it may periodically flow in a reversed direction for a short distance along the veins (Carus 1828: *Lycus* elytra; Stehr 1947: *Ephestia*). The flow direction may change depending on flow speed and flow pauses may occur (Carus 1828: *Tettigonia*; Brücke 1925: *Lampyrus* elytra; Brocher 1929: *Coccinella*- and *Galeruca* elytra; Yeager and Hendrickson 1934: *Periplaneta*; Thomsen 1938: *Calliphora* and *Musca*). Further support for the widespread occurrence of an oscillation mechanism in insect wings may come from reports of pulse pauses of accessory pulsatile organs (Brocher 1920: *Macroglossum*) and wing hearts (Thomsen 1938: *Musca*; Perttunen 1955: *Drosophila*). A more recent and detailed study on representatives of 14 insect orders stresses the existence of circulation in the wings (Arnold 1964). It contains the following passage which may, however, be considered as an argument that haemolymph oscillation is common in insect wings or that it may be superimposed on a circulation:

“Periodically in any insect, circulation in the wings may falter, reverse its direction of flow completely or in part, or stop entirely for short intervals. These periods of change tend to increase in frequency and duration with age, but they are normal occurrences at any age. They are unquestionably expressions of the unconfined nature of the circulatory system as a whole, and of inefficiency on the part of the mechanism for wing circulation.”

The differences in flow characteristics of the anterior and posterior wing veins in *Attacus* may explain why many authors must have gained the impression of a wing circulation in Lepidoptera, when visually

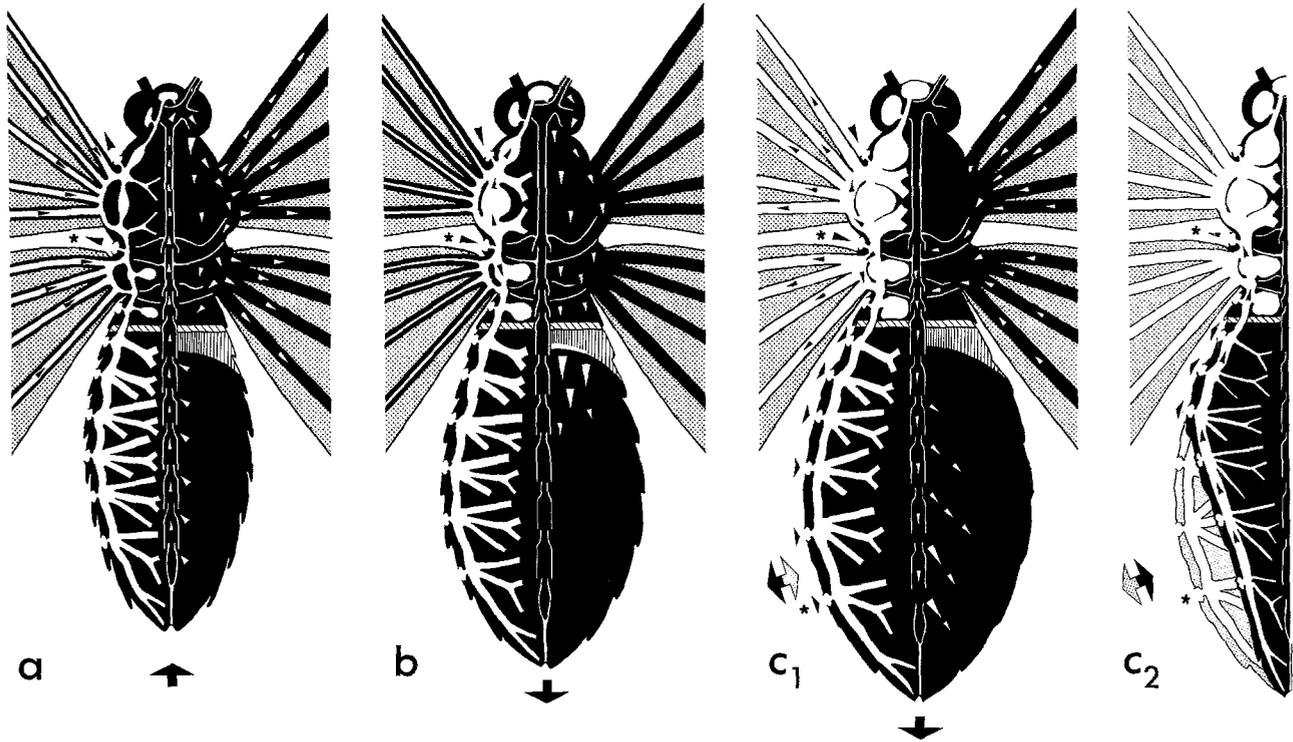


Fig. 14a–c. Interplay of haemolymph oscillation and tracheal ventilation in *A. atlas*. Right side shows haemolymph flow (white arrowheads), left side shows tracheal system and respiratory air flow (black arrowheads). Haemolymph flow in the body and air flow at the metathoracic and 7th abdominal spiracle (asterisks) are recorded in Wasserthal (1981). Air flow at other spiracles is based on unpublished data. Large arrows direction of abdominal movements; vertically shaded area is the ventral pressure valve behind the waist; a, b, c₁, c₂ moments of heartbeat sequence as indicated in Fig. 13

observing the movements of blood cells or stain particles: The haemolymph inflow is sudden and intensive, for a relatively short time, in the anterior veins, whereas in the posterior veins the outflow is more vigorous due to the more direct sucking effects of the POs. Supposing that the human eye recognizes only the quicker changes or that blood cells are moved only by vigorous flow, the inflow must be more conspicuous in the anterior veins, and the outflow in the posterior veins. This impression must be intensified by the earlier onset of inflow into the anterior vein. The pressure difference between the site of haemolymph influx in the anterior lateral thorax and the site of the sucking POs, to which the vein bases are exposed, may cause in net-veined wings, the inflow or outflow to follow a circular route.

In the wings with unelastic vein cuticle and absence of haemocoel in the wing membrane, the presence of an elastic cuticle *inside* the wing veins, the tracheal intima, is a prerequisite for the oscillating wing supply as shown in *Attacus*. Tracheae are quite common in insect wings (Whitten 1962). While the elytra of scarabaeid beetles contain rows of large tracheal air sacs which expand after volume decrease of wing haemolymph (Wasserthal, in preparation), the wing tracheae of many other insects have a rela-

tively small lumen (Arnold 1964), and their volume changes might be less effective for haemolymph transport. Some of these insects possess localised areas of elastic cuticle on the *outer* surface of the haemolymph sinuses, e.g. at the membrane folds of hindwings in beetles, along the flexible parts of the veins in some Diptera, the pterostigmas in dragonflies (own observations) and analogous haemolymph filled membrane spaces in other insect orders (Arnold 1963). Such membrane cuticles may be set under tension by negative pressure in the haemocoel. Thus, it can be hypothesized that they might also operate antagonistically against the pumping organs and exert some suction effect on the wing haemolymph.

In addition to its transport function the negative pressure in the wing haemocoel offers another important advantage: If the delicate wings are injured, e.g. by birds, there is no danger of significant haemolymph loss or even of bleeding to death, and the wing supply is maintained even if all wing membranes between the veins are lacerated from the outer margin. Lepidoptera, which have been maltreated in this way, can survive for weeks. In the convergently evolved split wings of the 'feathered' Pterophoridae (Wasserthal 1974), Alucitidae and the genus *Cenoloba* wing haemolymph supply is still realized in spite of the

much reduced wing membrane. In these small Lepidoptera an oscillating haemolymph supply in the wing is structurally manifest.

One may ask if wing haemolymph oscillation is a relatively late specialisation of modern insects or if it is an early acquisition and a general characteristic of all Pterygota. Most data, which point to its existence, come from Lepidoptera and Coleoptera which are considered to be among the most successful recent insect orders with regard to species number and diversity of specialisations. According to fossil records it is, however, possible that an oscillating mechanism is not restricted to holometabolous or neopteran insects but had already evolved in early Pterygota. In the oldest Palaeodictyoptera each of the serially arranged veins must have been supplied from its own basis (Kukalova-Peck 1978). Their primitive wings even lacked such structures which have been regarded to be important passages for wing circulation in recent insects, such as the sinus which encircles the outer margin and the axillary cord, the main exit for wing haemolymph (Arnold 1964). The 'particular refluxing', which Arnold (1964) has mentioned as a characteristic irregularity in mayflies (which are representatives of palaeopteran insects) is regarded by Kukalova-Peck as "a plesiomorphic reflection of the primitive type of circulation through serial veinal systems". Although haemolymph oscillation in the wings may be considered to be a predisposition for all recent pterygote insects, it has evolved to a highly regular supply mechanism in Lepidoptera. The consideration of 'irregularities' in the wing circulation as 'expressions of inefficiency' (Arnold 1964) does not meet the conditions, since the unconfined nature of the 'circulatory' system in connection with haemolymph oscillation represents a very efficient supply mechanism for utilizing even a small haemolymph volume for hydraulic functions.

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References

- Arnold JW (1963) A note on the pterostigma in insects. *Can Entomol* 95:13-16
- Arnold JW (1964) Blood circulation in insect wings. *Mem Entomol Soc Can* 38:1-60
- Brocher F (1920) Étude expérimentale sur le fonctionnement du vaisseau dorsal et sur la circulation du sang chez les insectes. III Le *Sphinx convolvuli*. *Arch Zool Exp Gén* 60:1-45
- Brocher F (1929) Observation physiologique sur la circulation du sang dans les ailes et dans les élytres chez la Coccinelle. *Rev Suisse Zool* 36:593-607
- Brocher F (1931) Le mécanisme de la respiration et celui de la circulation du sang chez les insectes. *Arch Zool Exp Gén* 74:25-32
- Brücke ET von (1925) Die Bewegung der Körpersäfte. III. Insekten. In: Winterstein H (ed) *Handbuch der vergleichenden Physiologie*, vol 1/1. Fischer, Jena, pp 933-947
- Carus CG (1828) Nachträgliche Bemerkungen über den Blut-Umlauf in den Kerfen, insbesondere den Blut-Umlauf in vollkommenen betreffend. *Isis* 21:478-480
- Freiling HH (1909) Duftorgane der weiblichen Schmetterlinge nebst Beiträgen zur Kenntnis der Sinnesorgane auf dem Schmetterlingsflügel und der Duftpinselfäden der Männchen von *Danais* und *Euploea*. *Z Wiss Zool* 92:211-290 + pl XII-XVI
- Jones JC (1977) The circulatory system of insects. Thomas, Springfield, Illinois
- Kukalova-Peck J (1978) Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. *J Morphol* 156:53-125
- Perttunen V (1955) The blood circulation and the accessory pulsatile organs in the wings of *Drosophila funebris* and *Drosophila melanogaster* (Dipt., Drosophilidae). *Suom Hyönt Aikak* 21:78-88
- Richards AG, Korda FH (1950) Studies on arthropod cuticle. IV An electron microscope survey of the intima of arthropod tracheae. *Ann Entomol Soc Am* 43:49-71
- Stehr G (1947) Beziehungen zwischen der Blutzirkulation im Puppenflügel und dem Zeichnungsmuster von *Ephestia kuehniella*. *Rev Suisse Zool* 54:573-608
- Thomsen E (1938) Über den Kreislauf im Flügel der Musciden mit besonderer Berücksichtigung der akzessorischen pulsierenden Organe. *Z Morphol Ökol Tiere* 34:416-438
- Verloren M (1847) Mémoire sur la circulation dans les insectes. *Mém Cour Mém Sav Étr Acad R Sci Belg* 19:1-93
- Vogel R (1912) Über die Chordotonalorgane in der Wurzel der Schmetterlingsflügel. *Z Wiss Zool* 100:210-244 + pl VII-VIII
- Wasserthal LT (1974) Funktion und Entwicklung der Flügel der Federmotten (Lepidoptera, Pterophoridae). *Z Morphol Tiere* 77:127-155
- Wasserthal LT (1976) Heartbeat reversal and its coordination with accessory pulsatile organs and abdominal movements in Lepidoptera. *Experientia* 32:577-578
- Wasserthal LT (1980) Oscillating haemolymph 'circulation' in the butterfly *Papilio machaon* L. revealed by contact thermography and photocell measurements. *J Comp Physiol* 139:145-163
- Wasserthal LT (1981) Oscillating haemolymph 'circulation' and discontinuous tracheal ventilation in the giant silk moth *Attacus atlas* L. *J Comp Physiol* 145:1-15
- Weber H, Weidner H (1974) *Grundriß der Insektenkunde*. Fischer, Stuttgart
- Whitten JM (1962) Homology and development of insect wing tracheae. *Ann Entomol Soc Am* 55:288-295
- Wigglesworth VB (1972) The principles of insect physiology. 7th edn. Chapman and Hall, London
- Yeager JF, Hendrickson GO (1934) Circulation of blood in wings and wing pads of the cockroach, *Periplaneta americana* L. *Ann Entomol Soc Am* 27:257-272
- Zeller H (1938) Blut und Fettkörper im Flügel der Mehlmotte *Ephestia kuehniella* Zeller. *Z Morphol Ökol Tiere* 34:663-738