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Haemolymph flows in the wings of Pierid butterflies visualized by vital staining (insecta, lepidoptera)

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Summary. The flow of stained haemolymph was photographed in the wings of resting *Pieris rapae, Pieris brassicae,* and *Gonepteryx rhamni* under UV-radiation at definite intervals after abdominal application of fluorescent tetracycline. There is no circular route in the wing. All wing veins are supplied with stained haemolymph from their own bases without preference to single veins. In freely resting *Pieris* with intact wings, most veins are completely stained after 20 min. The staining pattern supports the existence of an oscillating haemolymph supply mechanism in the wing veins and shows that the cross vein and encircling sinus are not essential in the supply of the longitudinal veins. Inflow of stained haemolymph into the wing membrane begins about 1 h after application and is generally completed within 12 h in *Pieris*. The wing membrane is supplied with fluid by diffusion and – especially under low relative humidity – additionally by haemolymph substitution of evaporated water.

This mechanism is associated with the disadvantages of water loss and probably salt withdrawal from the body. The puddling behaviour of butterflies might help in restoring these postulated deficits. It is hypothesized that haemolymph substitution of water evaporated from the wing membrane is a preadaptation for accumulation of defensive toxins and pheromones in the wing membranes, especially in diurnal and basking Lepidoptera.

The veinal system of 5-day-old young summer specimens of *Gonepteryx* stains more intensely than that of 4–5-month-old specimens just before entering hibernation. The transition of stained haemolymph from vein to membrane is reduced in this species, probably as an adaptation for water retention during diapauses.

A. Introduction

The haemolymph supply of fully developed insect wings has been visualized by autoradiography in *Lucanus cervus* L. (Lüdicke 1952) and in Odonata

(Münchberg 1966). These autoradiographs, however, give no information about the haemolymph movements and flowing routes within the wings. Kolyer (1973), who stained wings of *Pieris rapae* by applying neutral red, observed an inflow into the wing veins and therefore favoured the hypothesis of a gradual *efferent* haemolymph flow in the wing channels. In the wing veins of the giant silk moths *Attacus atlas* (L.) a periodic in- and outflow of haemolymph has been deduced from heat-marking experiments and from recordings of changes in thermal conductivity at the surface of the wing veins (Wasserthal 1982). In this investigation it could be shown that most wing haemolymph is periodically removed from the wings into the body by the sucking force of the accessory pulsatile organs and that it is retransported into the wing veins by the tension of the intima of the wing tracheae, mainly during pausing of the accessory pulsatile organs. This oscillation mechanism (Wasserthal 1982) and the efferent flow hypothesis (Kolyer 1973) conflict with the generally accepted model of insect wing circulation, according to which the haemolymph enters the anterior veins and leaves via the posterior veins after having transversed the wing either through cross veins, the encircling outer wing sinus, or the wing membrane (cf. Arnold 1964). In the oscillation mechanism, however, such a transverse passage was considered not to be essential for in- and outflow of wing haemolymph. The haemolymph transport through the wing membrane, the cross veins and the encircling wing sinus could not be examined by contact thermography. In this paper vital staining experiments are performed to visualize the haemolymph flows especially in these parts of the wings.

B. Materials and methods

Pierid butterflies were preferred for the staining experiments, because they have translucent unpigmented wing cuticle and could easily be collected outdoors or be bred in the laboratory. They were examined at 21°–23° C and 65 ± 5% relative humidity (RH). They were frequently provided with a 10% sugar solution. Before and between the experiments they were kept in a flight cage at 90% relative humidity.

The haemolymph was stained with an aqueous solution containing 10% of the antibiotic pyrrolidino-methyl-tetracycline Reverin Hoechst (TC). Under UV-radiation the TC shows an intensive yellow fluorescence even after being diluted further about 10–20 times. The fluorescent tetracycline has proven to be innoxious and stabile within living invertebrates. Here it is bound to calcium without losing its fluorescent activity (Märkel 1975). For ensuring a natural passage of the stained haemolymph into the wing, the stain was applied at the posterior abdomen. In each specimen a droplet (0.5 µl in *Pieris rapae* (L.) and *Gonepteryx rhamni* (L.), 2 µl in *Pieris brassicae* (L.)) was dispensed on the dorso-lateral intersegmental fold between the 6th and 7th abdominal segments. After the membraneous cuticle was carefully perforated with a fine needle, the fluid was generally immediately sucked into the abdominal haemocoele. Since the wing scales hide the wing cuticle and partly show fluorescence or UV-reflection, they had to be removed by gently brushing the wings with paper felt before the photographs could be taken. The UV-lamp (Osram HQV 125 W) was about 30 cm distant from the wings. When using a 35 mm Leicaflex camera with an UV absorbing edge filter (Leitz K 530) and Ilford film FP4, the exposure time was 4 s at f = 2.8.

1. Observation of the inflow process

Thirty-five *Pieris rapae* and eleven *Gonepteryx* were descaled on the ventral surface of the left wings before TC-application. After a recovery pause of about 1–2 h in a dark room

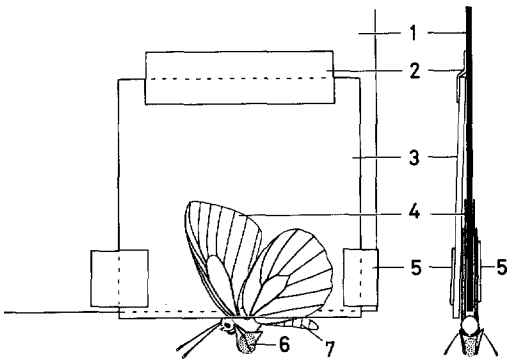

Fig. 1. Arrangement of a living specimen for continuous observation of stained haemolymph flows in the descaled wings. *1* dull black metal support, *2* hinge of adhesive tape for cover glass (*3*) which keeps the wings (*4*) in plane, *5* adhesive tape for attaching the cover glass and butterfly at the metal support, *6* paper felt for leg contact, *7* site of stain application

with 90% RH, the specimens were mounted at a vertical support (Fig. 1) with the body oriented horizontally and legs downwards. A light-weight ball of paper felt was given for leg contact to quieten the insects. A control photograph was made after mounting the specimen just before stain application, indicated by a 0 in the figures. This was necessary, because a slight fluorescence of the wing cuticle is subimposed on the TC-fluorescence. The staining process was observed and photographed at 1–15-min intervals within the first hour after incubation, and within the first day at intervals of 1–6 h. Thereafter, one photograph was taken each day or, in a few cases, once a week. Although the procedure of this staining series is very stressing for the animals, most of them tolerated the treatment and the TC and were capable of flying 1–6 days later. The five autumn specimens of *Gonepteryx* survived several weeks.

For quantitative analysis of the staining process the photo negatives of the veinal system of stained specimens were redrawn by projecting them at 15 times magnification. The changing positions of the stain front were indicated in each drawing with the corresponding time marks. For the measurements of the stained vein lengths each veinal stem including its branches was considered as 100% vein length, to which the stained veinal parts were related. Since the medial stem is obliterated in higher Lepidoptera and the medial branches are supplied via the radial or the cubital stem, the medial branches were treated in connection with these veinal stems and are not mentioned separately, differing from the usual terminology. The discoidal cross vein was treated by adding half of its length to the radius and half to the cubitus. The analis of the forewing, which was hidden under the hindwing, and the encircling sinus of the outer wing margin were not considered in the measurements.

2. Examination of the staining condition in intact Pieris rapae

Twenty specimens were kept undescaled after stain application. They could freely move in a cage under UV-illumination at about 65% RH. In ten of these specimens all wings were severed 20 min after incubation, in the other ten specimens 1 h after incubation. Immediately after wing cutting, the dorsal surface of the left wings was descaled under UV-radiation and photographed 10–15 min after severing. Then, the right wings were descaled and photographed together with the left wings 20–30 min after severing (Fig. 9).

3. Examination of the influence of the relative humidity upon the supply of the wing membrane

Undescaled, freely moving *Pieris brassicae* were incubated for 18–20 h. Two series, each consisting of 45 specimens with same age and sex ratio (20 males:25 females), were kept under different extremes of relative humidity: one series at 25% ± 5%, the other at 99% RH. Within the incubation time all specimens were individually fed two times and the specimens, kept at low humidity, were additionally allowed to suck water four times. After incubation, the wings were severed, descaled, and photographed (Fig. 10).

C. Results

1. The inflow of TC-stained haemolymph into the wing veins of restrained Pieris rapae and Gonepteryx rhamni with descaled wings

The time of first appearance of stain at the wing base varies between 30 s and more than 20 min (Figs. 2–7). About 50% of all specimens contain stained haemolymph in their basal veins 10 min after stain application. Staining of the complete veinal system is achieved after about 1–2 h in *Pieris* (Figs. 2, 3). In 5-day young *Gonepteryx* the staining of the complete veinal system needs 4–12 h (Fig. 4a). In 4–5-month-old autumn specimens of *Gonepteryx* the veins stain still more slowly and faintly and are completely stained only after 12–24 h (Fig. 4b). The appearance of the stained haemolymph in the wing veins is generally delayed in the descaled specimens and the stain advancement is slow in comparison with freely resting, undescaled specimens. The experimentally disadvantageous descaling prior to application was necessary for the visualization of the independent entrance of haemolymph into each single wing vein from its own base. The stained haemolymph becomes visible in the wing veins in different ways: The staining front may be sharp and appropriate for measurements (Figs. 2a, 3, 4a) or indistinct (Fig. 2b, 4b). In some specimens the speed of penetration is about the same in all wing veins (Figs. 2, 3b, 4a), in others the stained haemolymph runs ahead in the more posterior veins (Fig. 3a: cubitus) or in the more anterior veins (Fig. 3b: radius). The forewings are frequently stained earlier or more intensely than the posterior wings (Figs. 3a, 4b). The discoidal cross vein is stained via the cubital stem (Fig. 3a), via the radial stem (Fig. 9c) or via both veins (Fig. 2a). The encircling wing sinus receives stained haemolymph from all joining veins. Cuts of the margin lead to only local interruptions in staining of the outer sinus. In many specimens the stain forms phasic accumulations some millimetres from or at the distal ends of the veins, beginning about 1 h after TC-application (Figs. 2b, 3, 4b). In the course of the following hours the staining intensity decreases along the basal veins in some specimens (Figs. 3a, 4b).

A quantitative analysis of the staining process in single specimens shows that the staining front does not advance continuously but stepwise and synchronously in all measured wing veins (Fig. 6). The mean values of stained vein lengths suggest that the anterior veins (Sc, R) of the forewing and the posterior vein (A) of the hindwing achieve complete staining more rapidly than do the other veins (Fig. 6).

After complete staining of the veinal system in *Pieris* at about 1–2 h incubation, the stain slowly passes over from the veins and the encircling sinus into the wing membrane (Figs. 2, 3, 8). There is no staining gradient from the anterior toward the posterior wing membrane, neither in intensity nor in time. The staining of the marginal wing membrane remains incomplete in comparison with specimens which were descaled after incubation (see below). In the wing membrane of all *Gonepteryx rhamni* no stain was detectable even after days, although the membrane was not desiccated.

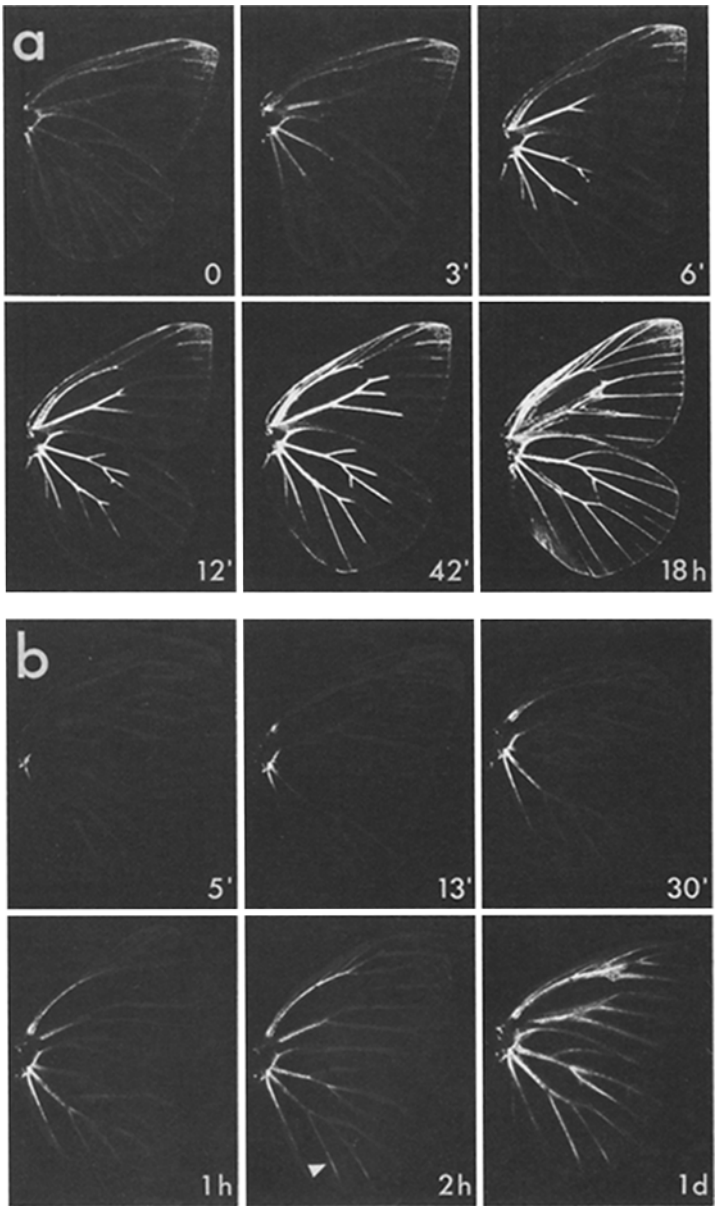

Fig. 2a, b. Simultaneous inflow of stained haemolymph into all veins of the descaled wings in restrained *Pieris rapae* after abdominal stain application at time 0. **a** Stained haemolymph advances relatively quickly with distinct front. **b** Stained haemolymph flows slowly and inconspicuously. Note phasic stain accumulation after 2 h (*arrowhead*)

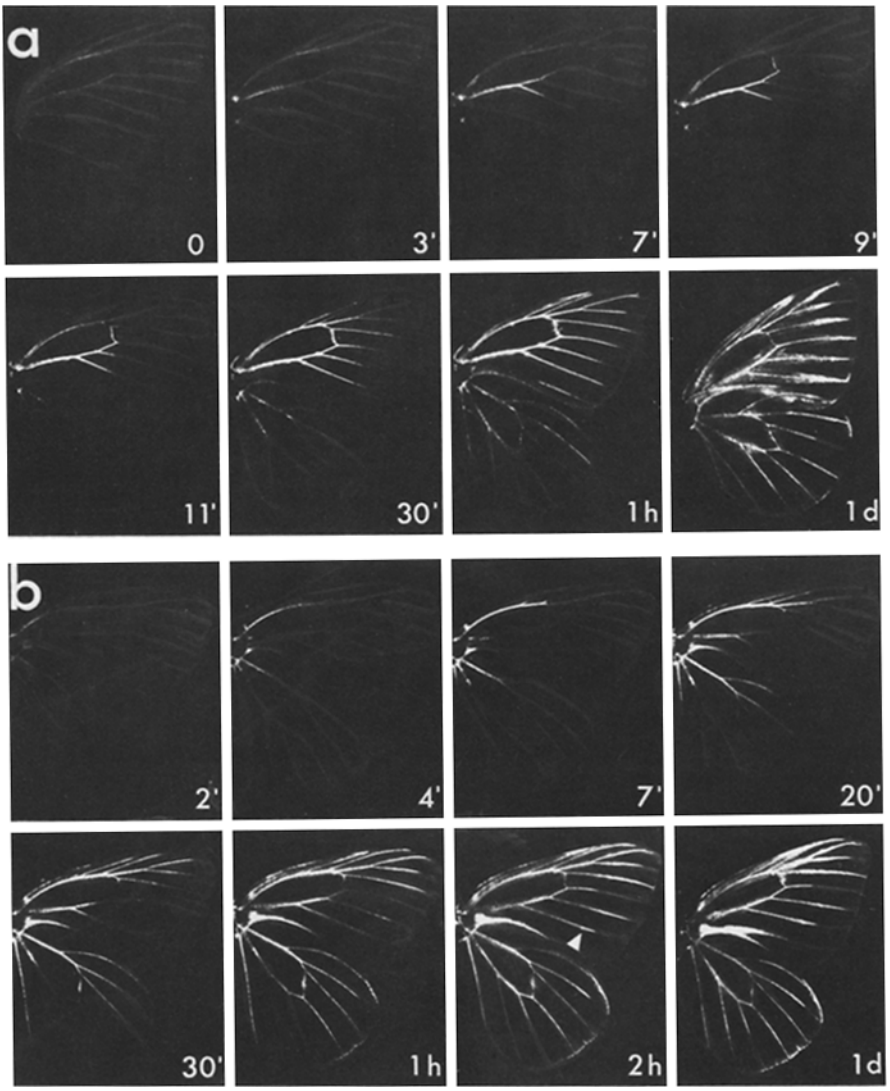

Fig. 3a, b. Unequal inflow of stained haemolymph into the descaled wings of restrained *Pieris rapae*. **a** Stained haemolymph runs ahead in a posterior (*cubital*) vein of the anterior wing and is delayed in the posterior wing. Note decrease in staining intensity along the basal veins of the anterior wing and stain accumulation at the distal veins after 1 d. **b** Stained haemolymph runs ahead in an anterior (*radial*) vein of fore- and hindwing. Note the phasic stain accumulation after 2 h (*arrowhead*)

2. Haemolymph flow in the wing veins of unrestrained Pieris rapae

The wings of specimens, which were descaled after TC-incubation, showed similar stain distribution but also individual variation in staining intensity (Fig. 9). The staining pattern of the left and right wings within most individuals is symmetrical. Differences in the staining pattern between left and

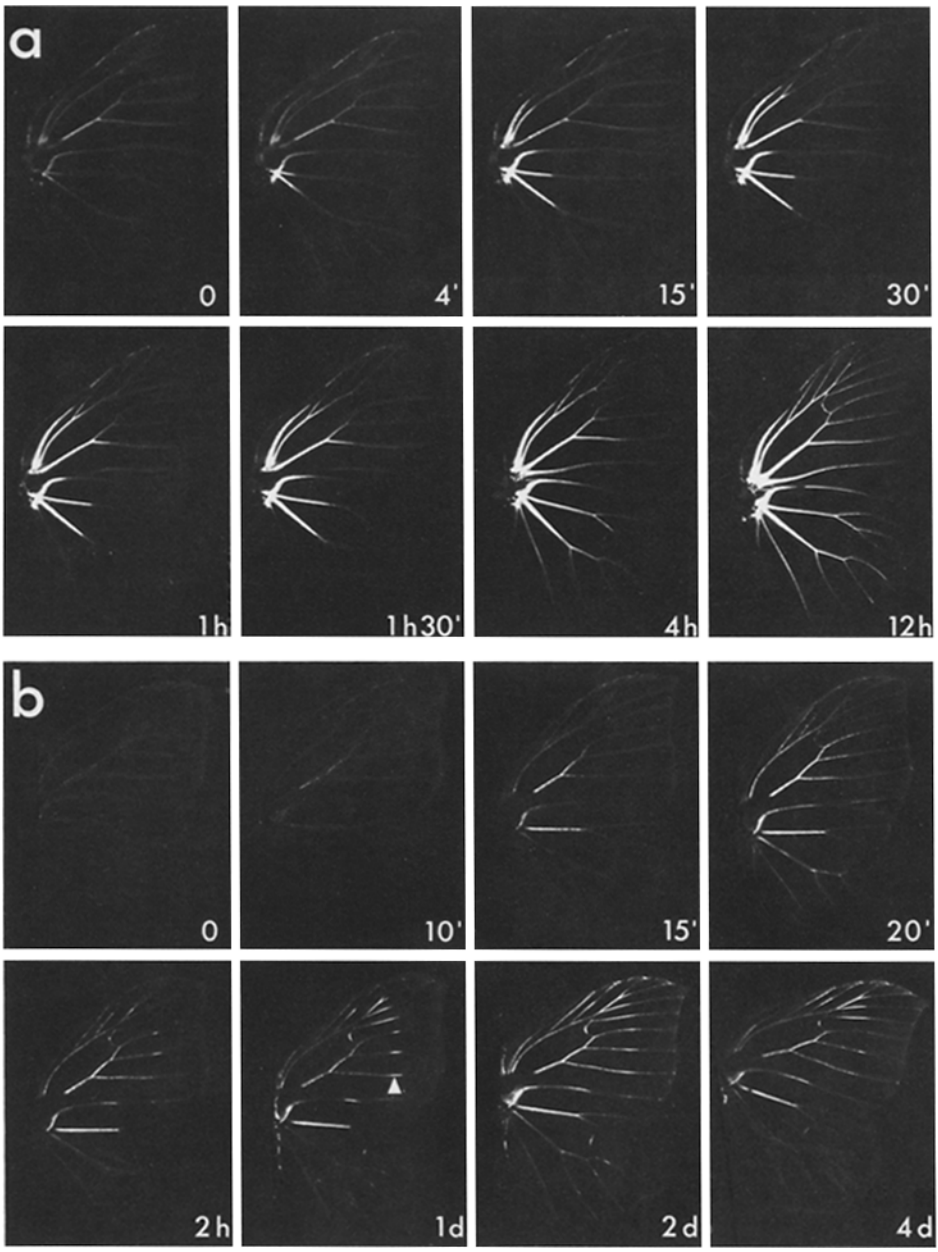

Fig. 4a, b. Inflow of stained haemolymph into the wing veins of restrained *Gonepteryx rhamni*. **a** Summer specimen 5 days after eclosion: Intensive staining of veins without staining of the membrane even after 12 h. **b** Autumn specimen about 5 months after eclosion, just before hibernation: Accumulation of stain phases along half length of the vein branches (*arrowhead*) and reduction of staining intensity along the basal veins 1 day after stain application

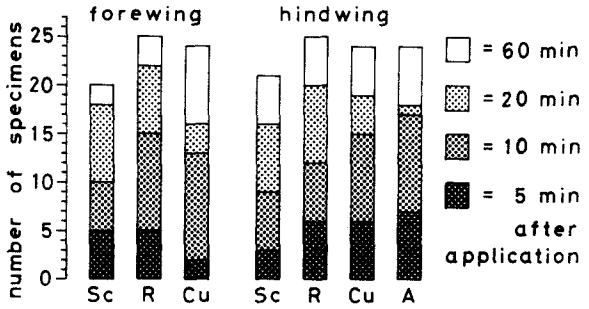

Fig. 5. Interval between application and first appearance of stain in the wing veins of 27 restrained *Pieris rapae*

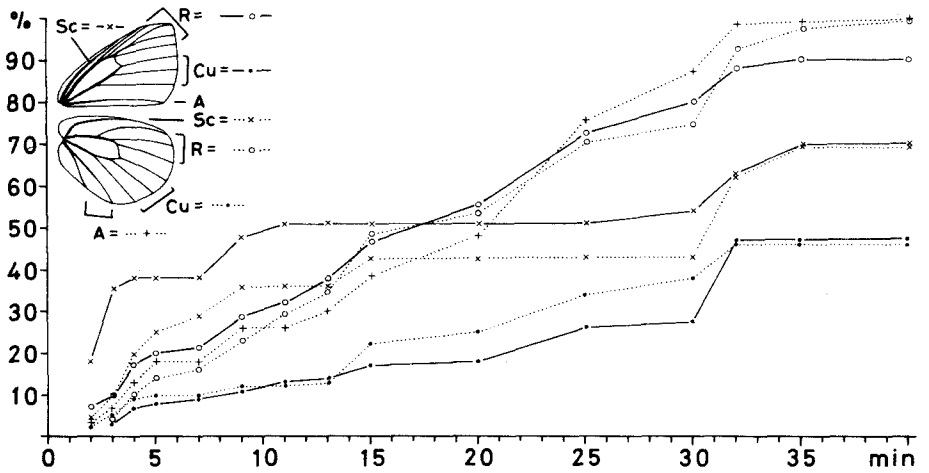

Fig. 6. Percentage of stained length of different vein stems with their branches in relation to time after stain application at 0 of a restrained *Pieris rapae*. Note the steplike advancing of stain after 7 and 30 min

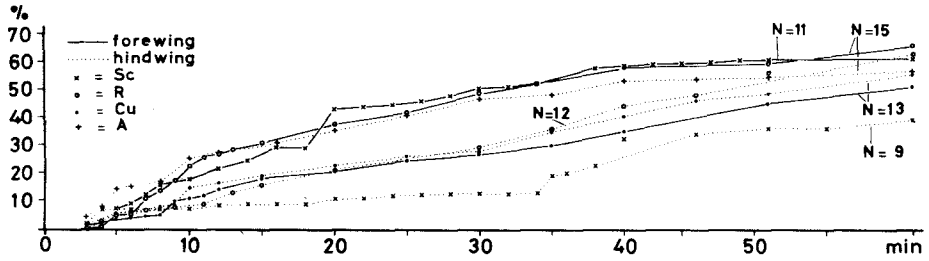

Fig. 7. Mean values of stained vein length (in percent) relative to time after stain application of restrained *Pieris rapae*

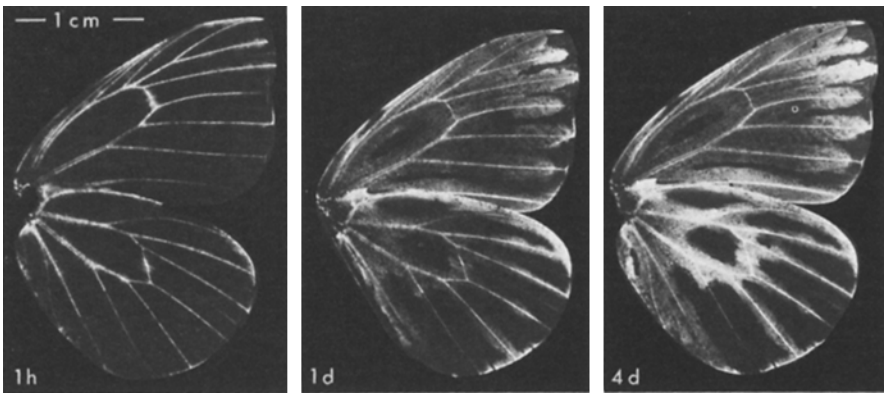

Fig. 8. Passage of stained haemolymph from vein into wing membrane of restrained *Pieris rapae* at 65% RH. Note accumulation of stain in the distal membrane of the anterior wing at 4 days after application. The incomplete staining of the outer margin is caused by slight damage of the encircling sinus by the descaling procedure prior to stain incubation

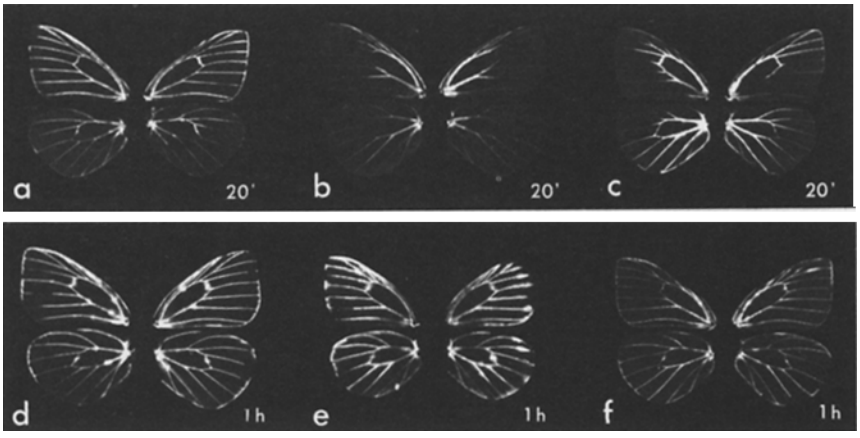

Fig. 9a–f. TC-stained wings of *Pieris rapae* which had rested freely after stain application. In *a–c* the wings were severed and descaled 20 min, in *d–f* 1 h after stain incubation

right wings result from defects like buckling of the veins (Fig. 9a: right forewing) or cuts of the lateral or posterior margin (Fig. 9e: right forewing). In wings, which were severed 20 min after incubation, most veins were stained along their entire length. In most specimens the discoidal cross vein and at least parts of the outer and posterior margin were stained already after 20 min (Fig. 9a). In some specimens the discoidal cross vein and the margins were still unstained (Fig. 9b), demonstrating an independent supply of the veins from their own base without a circular route. After 1 h incubation all haemocoele lacunae of the wings contained the fluorescent tetracycline (Figs. 9d–f) and in most specimens the beginning of stain transition from the lacunae into the wing membrane is visible (Fig. 9d, e).

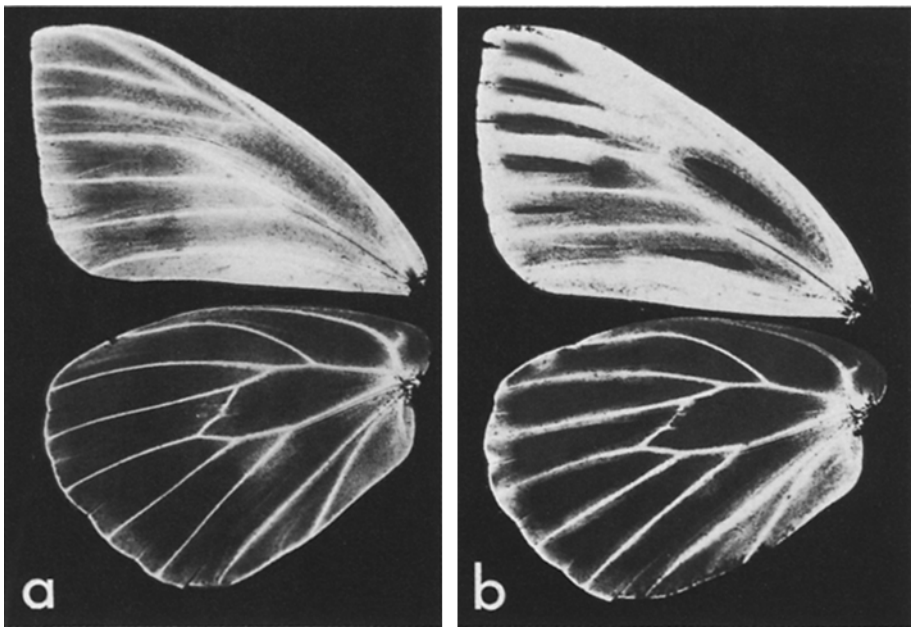

Fig. 10a, b. Typical differences in staining pattern of the wing membrane of *Pieris brassicae* related to high (**a**) and low (**b**) humidity of ambient air during stain incubation. Wings descaled and photographed 20 h after stain application. **a** At 99% RH and 28° C, total membrane area of the forewing is stained. Gradual decrement between the veins and the less stained membrane suggests stain diffusion. **b** At 25% RH and 28° C, membrane of the forewing shows intensely stained areas besides the veins and unstained areas more distant from the veins: While evaporated water is substituted by stained haemolymph beside the veins the more distant membrane areas are insufficiently supplied with fluid and desiccated

3. Influence of extremely high and low relative humidity on haemolymph supply in the wings of unrestrained, intact Pieris brassicae

For examination of the supply mechanism in the wing membrane, undescaled specimens were TC-incubated about 18–20 h, one series at 99% RH and another series at 25% RH. In both series the veinal system exhibited a similar staining pattern and intensity and is principally not different from the staining results at about 65% RH in *Pieris rapae*. The anterior wings frequently showed a still more intensive fluorescence than the posterior ones. Under both extreme RH-conditions, the wing membranes showed fluorescence, but exhibited a characteristically different staining pattern: At high RH the stain was distributed rather equally over the membranes with a continuous decrement from the veins and encircling sinus towards the more distant membrane areas (Fig. 10a). At low RH the staining intensity was conspicuously higher in the membrane areas bordering the veins, while the more distant membrane areas were desiccated and unstained (Fig. 10b).

D. Discussion

1. Supply of the wing haemocoele

The in vivo staining experiments show that all wing veins are supplied with haemolymph from their own base. The encircling sinus at the outer margin and the discoidal cross vein receive haemolymph from all veins which communicate with them. Thus they do not have the key function of a cross connection between anterior and posterior veins in Lepidoptera as has been suggested in the circulation model (Arnold 1964). The TC-marked flow into the veins corresponds to results from vital staining experiments in *P. rapae* (after feeding, injection or immersion, using neutral red) which demonstrated a stain flow from the body into the wing channels (Kolyer 1973). Kolyer observed no effects which could point at a retransport of wing haemolymph into the body. He therefore discussed the possibility of a pure unidirectional efferent vein supply. The present results with the TC-staining technique, which allowed a continuous and prolonged observation of the haemolymph-marking process, showed that the stained haemolymph, after once having entered the wing veins, does not simply persist in the vein channels but the fluorescent intensity rises near or at the vein ends whereas it decreases along the basal veins after the first day. These changes in staining intensity suggest that the haemolymph of the basal wing veins is washed out and is gradually replaced by less stained body haemolymph, while at the distal front the stain is accumulated. These phenomena are, however, not very conspicuous because the TC (like the neutral red) not only stains the haemolymph but also the tissues.

Kolyer (1973) explained the efferent haemolymph flow in the wing channels by water deficit created by evaporation. The TC-staining patterns and also the neutral red staining experiments of Kolyer can, however, be easily understood on the basis of the oscillation supply mechanism which has been shown to occur in moth wings and has been postulated for butterflies (Wasserthal 1980, 1982): The fluid deficit in the wing channels results from the small haemolymph content in the adult insect as a consequence of post-ecdysial diuresis (Nicolson 1976) and the suction activity of the accessory pulsatile organs which periodically further evacuate the vein haemocoele (Wasserthal 1982). The age-dependent and periodical haemolymph reduction in the wing haemocoele by heartbeat reversal is assumed in Pieridae also to be compensated by expansion of the vein tracheae which are set under tension as in the moths: it is this tracheal tension and the resulting negative pressure in the vein haemocoele which cause the observed centrifugal haemolymph flow in the wing veins. The complete staining of the wing veins at high humidity of ambient air demonstrates that evaporative water loss is not the decisive factor for the efferent haemolymph flow.

In *Papilio machaon* L. the accessory pulsatile organs, which are sucking the haemolymph periodically from the wings into the thorax haemocoele, show intermittent pulse activity with pulse periods and pauses occurring in the range of 2–9 min (Wasserthal 1980). Although the staining techniques

are not optimal for visualizing such a periodicity of haemolymph oscillation in the wings, the stepwise advancement of the stain front, which became visible in some specimens, when the intervals between successive photographic exposures followed closely enough, is consistent with a periodic supply mechanism also in Pierids.

The partial staining of the veins in descaled wings might be interpreted as indicating an incomplete and ineffective haemolymph exchange in the course of each period of haemolymph return into the wings. This is, however, disproved by the results in undescaled specimens. Here the entire veinal system is filled completely with stained haemolymph after 20 min. This staining speed is comparable with the neutral red staining experiments in *Pieris rapae* where the dye reached the wing margin within 10 min (Kolyer 1973). In the alae and elytrae of *Lucanus cervus* radioactivity appears 40 min after oral uptake of a $Na_2H^{35}PO_4$-sugar solution (Lüdicke 1952). Considering the indirect mode of application, a similar speed of haemolymph supply can be expected in this beetle.

The individual variability in onset and intensity of staining of the wings probably depends on the degree of mixing of the original TC-solution with the haemolymph after application. If the stain solution is immediately transported toward the wing bases by the forward beating heart, the staining intensity is expected to be greater. If the stain is first distributed and diluted in the abdomen during pauses or backward pulse periods of the heart, the staining of the wings should be delayed and less distinctive. The more intensive staining of the forewings in *Pieris* and the more rapid achievement of complete staining of its anterior veins (Sc, R) may be explained by the favourable anterior position of this wing and vein bases with regard to the first arrival of stained haemolymph in the anterior thorax during forward pulse periods of the heart. This assumption is in accordance to the slight time lead of the haemolymph inflow into the anterior wing veins of *Attacus* moths (Wasserthal 1982).

2. Supply of the wing membrane

The few and distant longitudinal veins, with only one cross vein, result in relatively large wing membrane areas in most Macrolepidoptera. Since, in addition, this wing membrane is separated from the vein haemocoele by hypodermal cells and contains no haemocoele lacunae (Wasserthal 1982), its supply with haemolymph was expected to be difficult. The staining results in *Pieris* suggest that only a one-way transport from the veins towards the membrane takes place. Haemolymph exchange from the membrane towards the veins could not be shown under these experimental conditions. Regarding the slow staining process of the wing membrane, two different mechanisms for its generation are assumed: (1) Diffusion of molecules through the cells and intercellular spaces and (2) replacement of evaporated water by haemolymph through intercellular spaces. At high ambient humidity, when evaporation is practically eliminated, diffusion alone seems to be responsible for the dye transport. At low ambient humidity, the intensity

of staining is unexpectedly increased near the veins. It is concluded that the evaporated water at low RH is substituted by (stained) haemolymph. This mechanism should be enforced during basking.

A supply mechanism, maintained by evaporation, consumes a high amount of water and withdraws salts from the body. The lepidopteran specialization for the uptake of liquid food meets the water requirements. In addition to nectar sucking, many diurnal Lepidoptera suck water at puddles, riversides, and even dew (Hering 1926; Portier 1949). Many of these species additionally ingest salts and other substances from mammalian sweat, urine, faeces, or decaying animals (Downes 1973) and substances even from withered plants (Pliske 1975; Schneider et al. 1975; Edgar et al. 1979). Arms et al. (1974) interpreted the preferential uptake of Na by *Papilio glaucus* L. as a compensation for the Na deficient diet of the herbivorous Lepidoptera. This hypothesis is, however, not sufficient to understand why puddling and uptake of salts is less frequently observed in the females and almost unknown in most lepidopteran species with nocturnal activity, although their way of larval and adult feeding does not fundamentally differ. Most moths do not expose their wings to solar radiation and therefore evaporate less water from the wings. Mated females of the Nymphalids *Neptis rivularis* Scopoli and *Charaxes jasius* (L.), which could be observed in the field throughout several days, only rarely exposed their wing surfaces for basking and spent hours of the day hidden in the vegetation with wings closed, obviously to avoid the bothering males. In contrast to the mated females, the males exhibit their wing surface to the sun for courtship and territorial display thoughout their life-time. In the flight cage the males of *Charaxes* occupied the sites closest to the light source with the highest temperatures, whereas the females rested in more distant locations (own unpublished observations). This behaviour suggests a sex-specific evaporative water loss via the wing surface in these Lepidoptera, which may be more common in butterflies.

The unidirectional supply mechanism of the wing membrane gives rise to the following hypothesis: The lepidopteran wing membrane is preadapted to be an ideal site for deposition of excretions, defensive toxins or pheromones, as is indicated by the occurrence of scent patches (Müller 1877) and the high concentration of defensive toxins (Brower and Glazier 1975) in the wings of *Danaus plexippus*. Then, an unidirectional efferent haemolymph supply, as Kolyer (1973) assumed in connection with the vein supply, should dominate in supplying the wing membrane and should be superimposed on the oscillation mechanism in the veins.

3. Colouration and supply of the wing membrane

In basking butterflies the colouration of the wing surface should be of importance for radiation absorbing properties and hence evaporative water loss. Some pierid phenotypes of cold climates (holarctic or boreoalpine populations or springtime generations of *Pieris napi* (L.), and female *Pieris bryoniae* Hübner) are characterized by melanized scales, bordering the wing

veins, while they are white in the summer phenotypes (see Shapiro 1977). The melanization along the vein borders corresponds exactly to the area of intensive TC-staining in the *Pieris brassicae* (L.) which were kept at low ambient humidity. The black wing areas are assumed to absorb solar radiation better than the white surfaces. It is not probable that the wing colouration beyond the basal third of the wings plays a significant role for thermoregulation of the body (Wasserthal 1975). Thus, the black vein borders may serve for enhancing the transition of haemolymph into the wing membrane by increasing the evaporative suction as an adaptation to cold and wet climates. The similar colouration patterns of other Pierids and some Papilionids (e.g. *Papilio machaon, Papilio xuthus* L.) may be interpreted likewise.

4. Implications of the unidirectional haemolymph supply of the wing membrane

A permanent inflow of haemolymph into the wing membrane, maintained by water evaporation, must result in an accumulation of substances in the haemolymph, such as salts and organic compounds. Hyperosmotic conditions should arise and should create a disadvantageous milieu which in addition to the slow supply may contribute to the degeneration of the hypodermal cells in the wing membrane of aged Lepidoptera (Reichelt 1925). The accumulation of salts in the wing membrane might further enhance the influx of relatively hypo-osmotic vein haemolymph, but might also render the membrane hygroscopic. While the period of accumulation of many substances in the wing membrane occurs already during scale and wing development in the pupa, or is unknown, there is evidence that the toxic pheromone precursors (or enzymes) in the wing pouches of *Danaus chrysippus* are deposited during adult life (Boppré et al. 1978). It is conspicuous that wing membranes with scent patches are almost restricted to males of species with diurnal activity and basking behaviour, where they probably originated independently several times (Müller 1877).

5. Longevity and economic supply of the wing membrane in Gonepteryx rhamni

The practically unstained wing membranes of this species suggest that a haemolymph supply of the wing membrane must be almost absent. This is especially remarkable, because *Gonepteryx rhamni* is the European butterfly-species with the longest adult life: from July to June with a summer and a winter diapause. The capability of water retention must be highly developed in this species. *Gonepteryx* belongs to the laterally basking butterflies and very rarely exhibits basking behaviour. Evaporative water loss via the wings is therefore probably reduced. During activity the evaporated water can be replaced by nectar feeding. Additional water-(salt-) uptake by puddling at water- or salt-sources in this species has neither been de-

scribed in the literature nor observed by the author. More critical than the activity periods are the diapauses from August to September and from November to February, when the evaporated water cannot be restored by oral uptake. The reduced or absent supply of the wing membrane may thus be interpreted as an adaptation to spare water during these long periods of inactivity. The following problems, however, deserve to be solved: What is the structural basis for preventing the inflow of (stained) haemolymph from the veins into the wing membrane and to prevent evaporative water loss? Which mechanism keeps the wing membrane elastic? Is the wing membrane capable of absorbing water from the atmosphere?

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