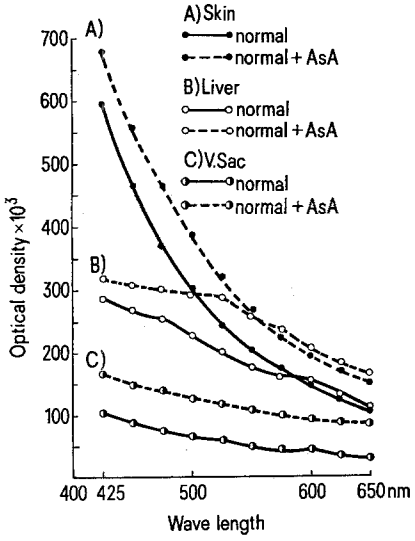
skin showed higher concentration of melanin pigments than the liver and vocal sac. Administration of ASA to normal toads produced an increase in melanin content of skin, liver and vocal sac compared to saline treated controls (Figure).



The effect of ascorbic acid (ASA) on the melanin content of toad skin, liver and vocal sac. Melanin content of A) normal and ASA treated toad skin, B) normal and ASA treated toad liver and C) normal and ASA treated toad vocal sac.

Discussion. The present study demonstrates that ASA causes a considerable increase of melanin pigments in the skin, liver and vocal sac of toads. The possible mechanism responsible for the action of ASA on melanin formation has not been elucidated. ASA in the testis[10] and other tissues (unpublished observation) of toads is known to be oxidized to dehydroascorbic acid. On the other hand dehydroascorbic acid helps in oxidation-reduction of cell by oxidizing the reduced glutathione[11]. It is generally agreed that the enzyme tyrosinase, which is responsible for melanin formation, is inhibited by reduced glutathione[12]. The presence of oxidized glutathione in buffered tyrosine-tyrosinase mixture increases melanin pigmentation[13]. Furthermore, dehydroascorbic acid is known to stimulate steroid hormone synthesis in toad testis[6]. FIGGE and ALLEN[13] have reported that local application of estrogen increases the skin pigments. They have suggested that the enzyme tyrosinase oxidizes ring A of estrone to a quinone which in presence of copper catalyst oxidizes the glutathione.

The possibility remains, therefore, that ASA in its oxidized form (dehydroascorbic acid) stimulates melanin synthesis by oxidizing glutathione either directly or indirectly by increasing sexhormones.

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Heartbeat Reversal and its Coordination with Accessory Pulsatile Organs and Abdominal Movements in Lepidoptera

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Summary. Haemolymph in certain Lepidoptera at rest is periodically transported from the anterior body to the abdomen and reversed by the coordinated activity of the heart, the accessory pulsatile organs and the abdomen. This oscillatory haemolymph transport is suggested to support haemolymph exchange and air ventilation in the anterior body and wings.

Although heartbeat reversal has been repeatedly described in insects[1–7], it is now generally regarded as being not essential for circulation, but rather a disturbance of heart automatism[6,8] or perhaps a stress reaction[5,9]. In some recent papers, which deal with the electrophysiology of heart rhythm in moths, reversal is either not mentioned[10,11] or is regarded as a rare and irregular event[12]. While mechanisms inducing and controlling heartbeat reversal were the central point of interest[2,5,6,13], little has been elaborated about its function[2,13,14].

Results. A new method, 'contact-thermography'[15] (Figure 1), allows one to examine free, resting insects during their whole lifetime without damage or narcotization, so that periodic heartbeat reversal could be shown to be a regular performance in pharate and adult moths (*Attacus atlas* L., *Argema mittrei* Guér., Saturniidae, Figure 1) and butterflies (*Caligo brasiliensis* Fldr., Brassolidae)[15]. Simultaneous recording of the heart, meso- and metathoracic pulsatile organs (PO's), which are responsible for the adduction of wing haemolymph[11,16]

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showed that these PO's pulsate intermittently with their activity periods mainly during backward beating and the pauses coincidental with forward beating of the heart (Figure 2). More precisely, an effective haemolymph convection by the PO's begins somewhat later than the backward periods and the pulsations extend into the heart's forward periods. Some time after the start of backward periods, peristaltic movements of the posterior abdominal part can generally be registered in all individuals, but they do not occur in every backward period (Figure 2). The beginning of the forward beating of the heart is generally accompanied by one slow abdominal contraction. The convective effect of this large contraction is perceptible even on the meso- and metascutellum. The haemolymph inflow in the anterior body thus happens much more vigorously than the evacuation. The presence of much more haemolymph in the anterior body during the forward periods is shown by the improved heat dissipation: despite the pauses in the PO's and constant haemolymph temperature, the equalized temperature level on the thorax remains below that of the backward periods.

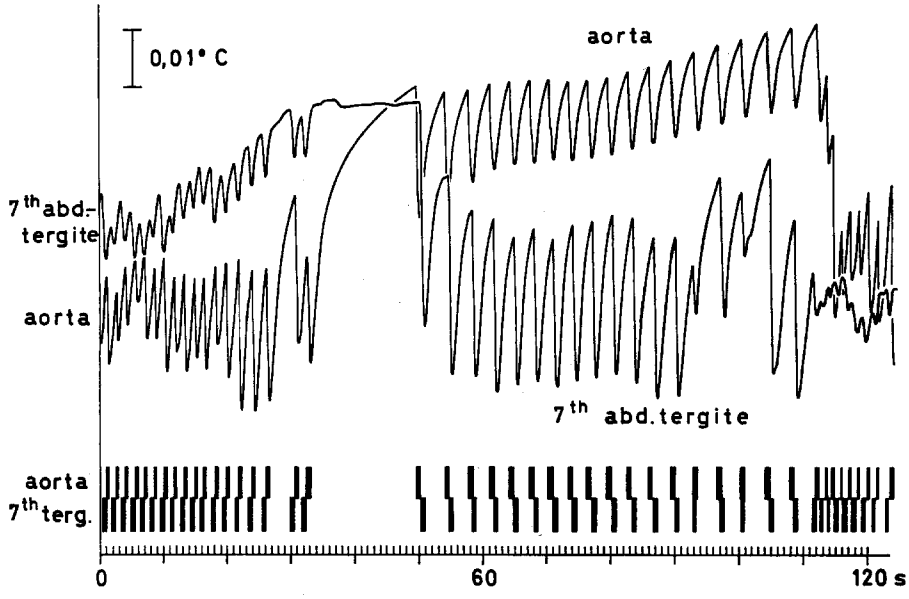

Fig. 1. Thermocardiograms of *Argema mittrei* (♂, 14 days old), representing the (convective) activity of the heart during forward and backward beating. Each thermogram is produced with one thermistor (diam. 0.1 to 0.25 mm), which at the same time serves for applying heat ($\Delta\vartheta$+ 1.8 °C) to the cuticle above the pulsatile organ and for the measurement of temperature differences: Each pulse removes the applied heat to an extent corresponding to its transport capacity. Each black bar represents the moment of convective cooling during heart contraction. In backward beating the contractions are first visible in the aorta.

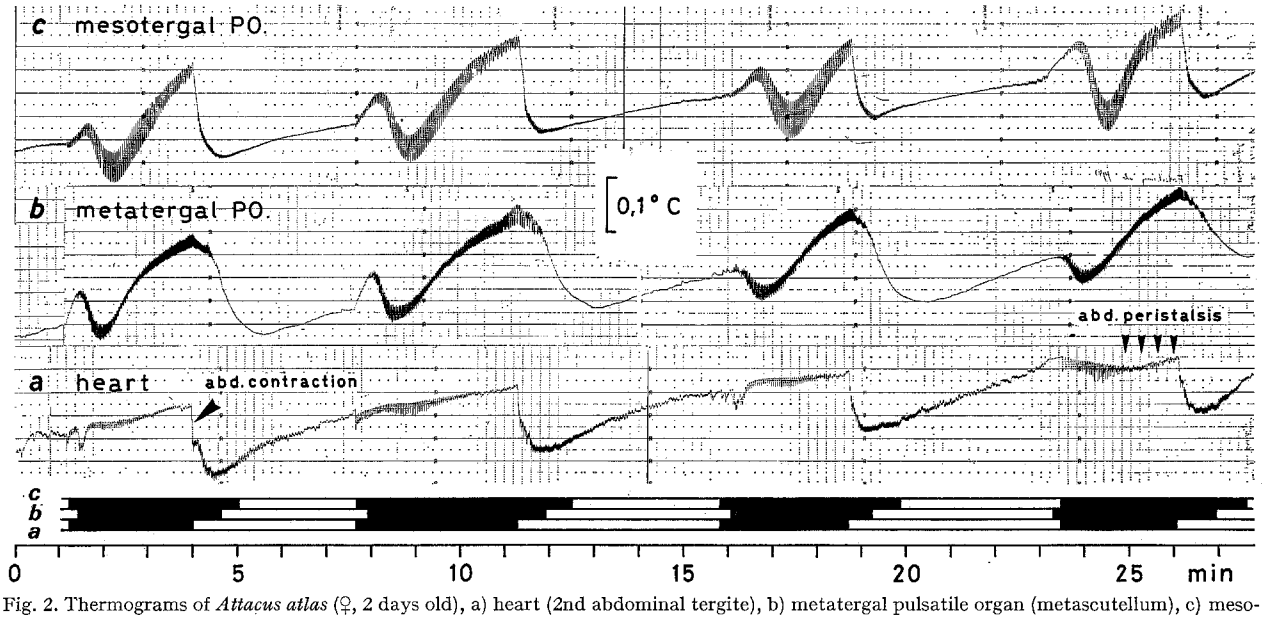

Fig. 2. Thermograms of *Attacus atlas* (♀, 2 days old), a) heart (2nd abdominal tergite), b) metatergal pulsatile organ (metascutellum), c) mesotergal pulsatile organ (mesoscutellum). The thermograms show the integrated convection beneath the measuring point. The pulses are superimposed on periodic slow waves of abdominal movements and alternations of heat dissipation, caused by (haemolymph-) mass changes. Black bands in a) backward periods, in b) and c) activity periods.

Discussion. The question is, how the evacuation of the wings takes place when they contain an excess of haemolymph after expansion (8 times normal in *Bombyx*[17], 5 times in *Attacus*) and how an efficient exchange of haemolymph with that from the abdomen is guaranteed in species with relatively large wings. The generally accepted model of circulation in the wings with afferent and efferent sinuses, joined to a longitudinal body circulation[8,16,18], would imply a short circuit wing supply: the haemolymph once sucked out of the wings by the PO's must be taken to the head by the aorta. It leaves the frontal sac and enters the thoracic cavities, whence it can enter the anterior wing veins again, only a part of it being exchanged. The delay in the start of transport activity of the PO's seems to be a key for the understanding of heartbeat reversal and the mechanism of wing supply. As the head and thorax become drained shortly after backward peristalsis has begun, there must be a haemolymph deficiency when the PO's start their activity. Thus the anterior aorta and the lateral thoracic passages to the PO's[16] do not compete with those from the wings, so that haemolymph can be sucked mainly from the latter; additionally no haemolymph can be available to enter the wing veins during backward periods. The wings must also become drained. One function of the haemolymph oscillations, instead of a pure circulation, would be to accomplish an effective exchange in the wings containing relatively large amounts of haemolymph. The correlation of backward pulsations to the quantity of wing haemolymph becomes obvious in *Caligo*, where the duration of backward periods is increased after wing expansion[15]. The to and fro movements of blood cells observed in the wings, especially the pupal wings of *Ephestia*[19], show that similar conditions may exist in Microlepidoptera.

When the wing haemolymph becomes reduced after wing expansion, the soft cuticular surfaces can give way and approach one another, while the veins in fully developed adults are rather sclerotized. Changes in haemolymph pressure are thus presumed to be compensated by the large tracheae in the wing sinuses and by the airsacs of the thorax and head, which must expand by haemolymph evacuation during the backward periods. The wing tracheae must contract under their own elasticity as soon as haemolymph flows in from the anterior body at the beginning of the forward period. The activity of the PO's on the one hand, and the elasticity of the tracheae on the other, would thus be antagonistic forces for air- and haemolymph ventilation in the wings, supported by coordinated directional changes of heart peristalsis and abdominal movements.

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Immunohistological Investigations of N-Acetylserotonin in the Rat Cerebellum after Parachlorophenylalanine Treatment

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Summary. The amount of N-acetylserotonin (NAS) in the granule layer of the rat cerebellum was investigated using immunohistologic double antibody technique. After 5 days of treatment with parachlorophenylalanine (PCPA) an increase of NAS was observed. The possibility of a differential effect of PCPA on serotonin synthesis in the neurons and the nerve terminals is discussed.

N-acetylindolealkylamines (NAI) are derivatives of serotonin. The two best known NAIs, melatonin and its precursor N-acetylserotonin (NAS) were first identified in the pineal gland[2], however, subsequently were localized in our laboratory in the cerebellum, retina[3], hypothalamus, Harderian gland and the lower brain stem[4] using immunohistological double antibody techniques. The first antibody developed by Grota and Brown[5] did not distinguish between NAS and melatonin. However, crossreactivity to other derivatives of serotonin was only minimal (to serotonin only 0.02%) and to other hormones and neurotransmitters essentially no crossreactivity was found[3]. Recently a new type of antibody was developed which is almost exclusively specific to melatonin (Table I). We have now used this antibody to reexamine tissues in which NAIs were previously seen. The new antibody did not induce any specific staining in the granule layer of cerebellum (Figure 1). On the other hand in tissues where synthesis of melatonin in vitro has been shown[6] and in the Harderian gland where melatonin has been identified by gas chromatography-mass spectrometry (J. Warsh, personal communication) positive staining was seen[4]. On the basis of these findings and the known crossreactivity of the NAI antibody to NAS and melatonin we now conclude that the previously identified NAI in the granule layer of cerebellum[3] is NAS. In order to check the relationship of NAI positive substance to serotonin, we have chosen to treat experimental rats with parachlorophenylalanine (PCPA). PCPA is a blocker of tryptophan-5-hydroxylase (TR-5-OHase), an enzyme essential in the synthesis of serotonin from tryptophan (Table II). The brain of animals treated for 3–4 days with PCPA exhibits only 15–20% of the original amount of serotonin[7],

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