

MECHANISMS OF BODY-TEMPERATURE REGULATION IN HONEYBEES, *APIS MELLIFERA*

I. REGULATION OF HEAD TEMPERATURE

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SUMMARY

1. Honeybees when endothermically heated maintained an elevated head temperature (T_H). During free flight at 17 °C T_H was approximately 7 °C above ambient temperature (T_A). However, during flight at T_A near 46 °C, head temperature averaged 3 °C below T_A .

2. When tethered bees were heated on the head till T_H reached 46 °C, they regurgitated nectar from their honeycrop, held it on their 'tongue', and initiated violent aortic pulsations in the head and in-out movements of the nectar droplet. Temperature changes in the head corresponded with heart pulsations. Head temperature was prevented from rising and was stabilized.

3. The heart pulsations in the abdomen and aortic pulsations in the head (and abdominal ventilatory movements) were often synchronous, but during heating of the head they were often independent of each other in both frequency and amplitude.

4. The fluid droplet was several degrees Centigrade below head, thoracic and ambient temperatures, and it remained in motion in and out of the body. It caused T_H to be 4-8 °C lower, by the resulting evaporative cooling, than is possible without the droplet.

5. It is concluded that at low T_A the elevation and regulation of T_{Th} automatically results in the elevation of T_H . However, at low T_H heat flows from the heated thorax to the head, not only by passive conduction but also by physiologically facilitated blood circulation.

INTRODUCTION

Studies on the physiology of body-temperature regulation in insects have been concerned almost exclusively with the regulation of thoracic temperature (Heinrich, 1974). The thorax contains not only the heat-producing muscles but also the ganglia that regulate their contraction. The neural output from the thoracic ganglia upon which flight control depends is highly temperature-dependent (Kammer, 1968; Hanegan, 1973). Additionally, a high thoracic muscle temperature is necessary to achieve the power output for flight (Leston, Pringle & Tamasige, 1962; Esch, 1976).

The insect head contains negligible muscle mass that could be used for endo-

thermic heat production. However, the anterior end of the aorta should release heated blood near the brain. (The blood would have been heated during its passage through the active flight muscles in the thorax.) Is the temperature of the head (and by implication the temperature of the neural control centres it contains) regulated in those insects which, like honeybees (Esch, 1960; Bastian & Esch, 1970; Heinrich, 1979*a*), regulate thoracic temperature (T_{Th}) over some ranges of ambient temperature (T_A)?

MATERIALS AND METHODS

Body temperatures of free-flying bees were examined in animals transferred directly from the entrance of an observation hive (having access to the outside) to a Modulab room whose temperature was controlled to ± 0.5 °C. Relative humidity (measured by wet-bulb temperature) varied from 14% to 40%. The bees flew toward the fluorescent lights at the ceiling, and they were nudged with a pencil to stimulate them to resume flight if they landed. In addition, continuous flight was also achieved (where indicated) by tarsectomizing bees. Tarsectomized bees were unable to land on the walls or ceiling. After the bees had been in continuous flight for 3–5 min they were grasped between thumb and forefinger of a gloved hand. Body temperatures were measured by inserting a 40-gauge thermocouple (threaded into the tip of a hypodermic needle with o.d. = 0.05 mm). Temperatures were read to the nearest 0.5 °C from an Omega Engineering Thermocouple Thermometer within 3 s of capture.

Heat would flow from the bee into the glove and the thermocouple when body temperature is elevated, and from the glove into the bee when T_B is below ambient. The amount of this passive heat flow, which is a direct function of the temperature difference and the duration of contact, constitutes a source of error that was estimated by taking body-temperature measurements of bees with arbitrary but known internal temperatures. Body temperatures were continuously recorded in live endothermic bees, and these bees were then grasped and probed with the thermocouple as under experimental conditions. Average measurements of T_H were 0.06 °C lower for every °C of head temperature excess ($T_H - T_A$), while T_{Th} measurements were indistinguishable from actual T_{Th} . Therefore T_H was actually slightly higher (< 1 °C) than when measured at the low T_A , and slightly lower (< 1 °C) at the high T_A , where T_H was below T_A . The thermoregulatory capacity here demonstrated is thus a conservative estimate of the bees' capabilities.

Heat transfer to the head was investigated in bees fastened on to a styrofoam pad with pairs of insect pins crossing between head and thorax and between thorax and abdomen. An incandescent lamp was used to focus a narrow beam of heat on to the thorax (or the head) while the head (or abdomen) was shielded with tin foil. Several body temperatures were simultaneously recorded with a Honeywell multi-channel potentiometric recorder, using 46-gauge copper-constantan thermocouples insulated with cotton, except for the tip. In some experiments heart and/or aortic activity were also concurrently recorded with body temperatures using a Beckman R411 Dynograph recorder. In some experiments, body temperatures were continuously monitored with the Omega Thermocouple Thermometer and recorded, along with heart or aortic activity, on the same chart of the Dynograph recorder, in

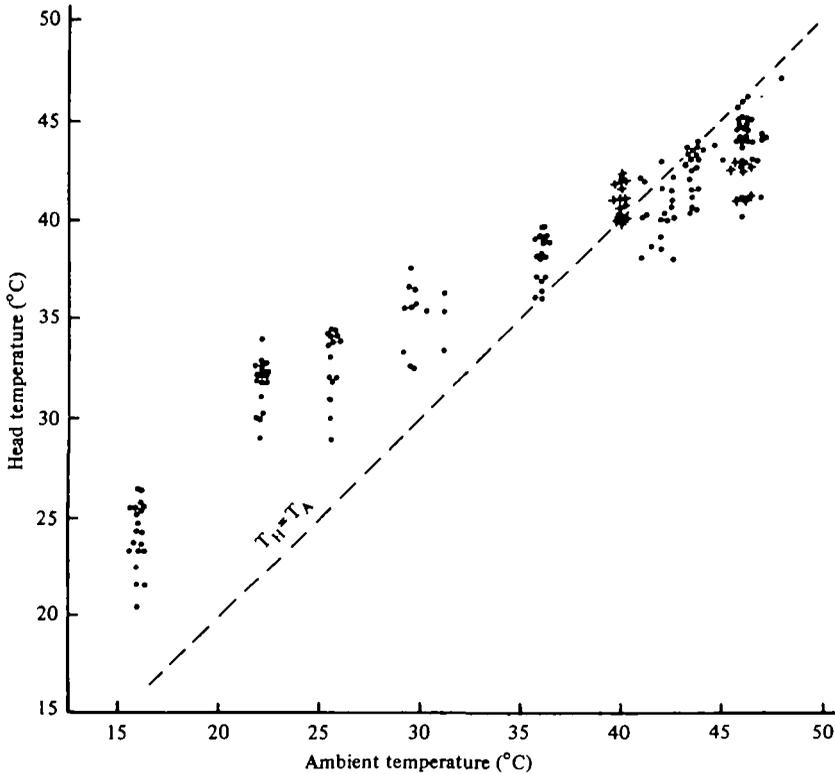


Fig. 1. Head temperatures during free flight as a function of ambient temperature. Crosses indicate T_H of tarsectomized bees.

order to provide a continuous record at high magnification, and to examine inter-relationships of events.

Mechanical activities of both the heart and the aorta were measured from paired electrodes on each side of the respective organs. Electrical resistance changes, resulting from mechanical disturbance or fluid flow between the paired 36-gauge copper electrodes, were measured using Biocom Model 2991 Impedance converter and recording with the Dynograph. The electrodes and thermocouples were inserted about 1 mm into holes pierced with an insect pin, and they were glued in place using a mixture of melted beeswax and resin applied from the head of an insect pin.

Operative procedures were done while the animals were under light CO_2 narcosis. The bees were generally not narcotized longer than 1 min. After coming out of CO_2 narcosis the bees were offered a 30% sugar solution. They generally fed. Measurements on each live bee were completed in 20 min or less. In order to distinguish active from passive heat transfer, bees that had been heated on the head (or thorax) while body temperature was monitored were then killed by injecting 1 μl ethyl acetate into the thorax. They were again heated (after 10–15 min) with the thermocouples and heat-lamp remaining in the same positions. The differences in body temperature changes between live and dead bees were used to determine the extent of active cooling and to differentiate passive from active processes of heat transfer.

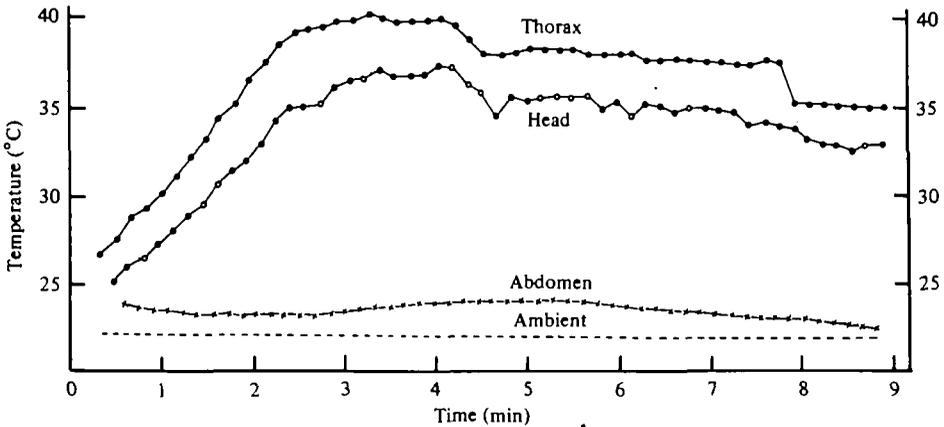


Fig. 2. Thoracic, head and abdominal temperatures of a bee during endothermic warm-up and stabilization of thoracic temperature. The bee was walking on a Styrofoam ball while suspended by the thermocouples.

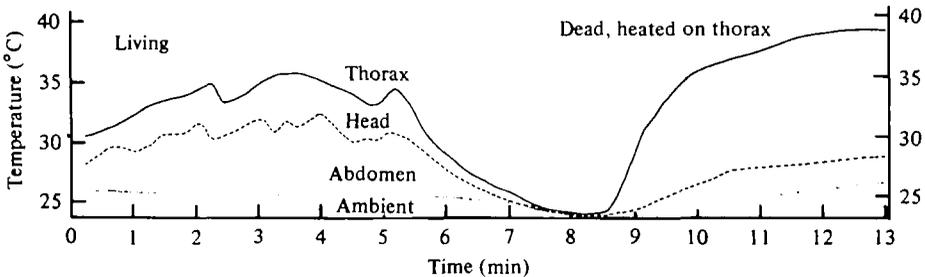


Fig. 3. Thoracic, head and abdominal temperatures of a bee while it was alive (left) and after it was dead and heated on the thorax.

RESULTS

(A) Head temperature

During free flight at T_A from 15 to 25 °C head temperature averaged 7 °C above T_A (Fig. 1). However, at 36 °C T_H averaged only 2 °C above T_A , and at $T_A > 40$ °C T_H was usually below T_A . At 46 °C the highest T_A at which the bees were tested in flight, T_H was up to 5 °C below T_A , averaging about 3 °C below T_A (Fig. 1). These data suggest that T_H is not a passive function of ambient temperature. Head temperature is physiologically regulated by being held < 45 °C at high T_A .

Is T_H actively or passively elevated at the lower T_A ? In bees with thermocouples implanted in both head, thorax, and abdomen, T_H was always close to but below T_{Th} . As in bees that had been in free flight, T_H generally rose and fell in parallel with changes of T_{Th} , while T_{Ab} remained close to T_A (Figs. 2 and 3). Nevertheless, T_H showed some independence from T_{Th} in that it sometimes decreased while T_{Th} increased (Figs. 2 and 3).

In bees that maintained endothermically elevated and stable body temperatures (at $T_A = 23$ –24 °C) while pinned down on to a Styrofoam pad, the average ratio in temperature excess of head ($T_H - T_A$) v. temperature excess thorax ($T_{Th} - T_A$) was

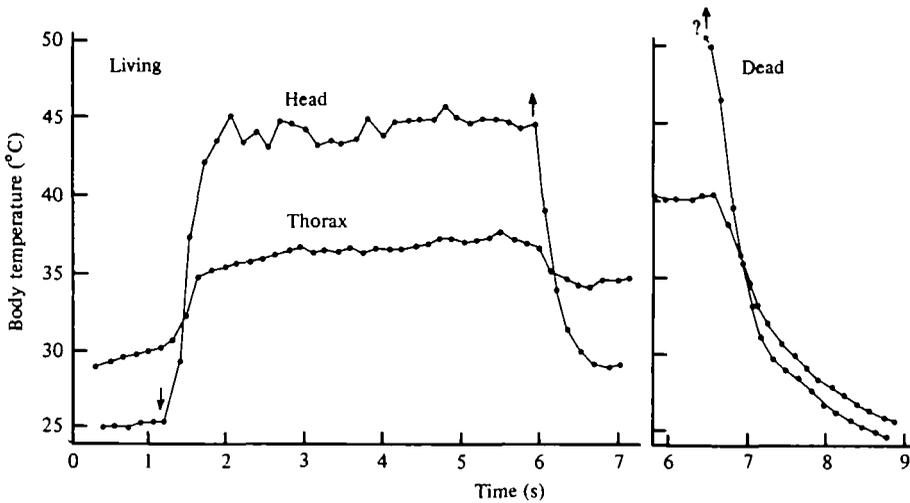


Fig. 5. Reduction and stabilization of head temperature in a bee heated on the head. The living bee (left) which stabilized T_H near 45°C received the same input of heat as after it was killed, when T_H exceeded 50°C (right). Also shown are stabilization of T_{Th} and T_B by endothermic heat production in the live bee after exogenous heating was terminated. Passive cooling of head and thorax of the killed bee are shown at far right. Heat was applied at downward pointing arrow and removed at upward pointing arrow.

The droplet could not be observed directly in bees during free flight. However, it was clearly visible in tethered bees flying in place at high T_A . No droplet was observed at any time during flight at room temperature or lower T_A . Bees in free flight at high T_A presumably also engaged in droplet extrusion, because their mandibles were usually open and their tongues were in motion ('tongue lashing') after landing. A droplet was visible when these bees, which had just been flying, were examined under the microscope.

Bees that had been 'soiled' with the regurgitated sugar solution from the honey-crop were cleaned by hivemates when they were caged together; their pile again became fluffy.

The regurgitated droplets showed large fluctuations in temperature (Fig. 4). Droplet temperature was always several $^\circ\text{C}$ below T_H , and the fluctuations in droplet temperature were not correlated with tongue movements alone. It was difficult to remove samples of the fluid for analysis because the bees rapidly retracted the fluid. It is probable that the droplet is kept in motion in and out of the body. It could thus take up heat in the body, and lose it by evaporation when it contacts the atmosphere.

(C) Head cooling

The appearance of a droplet in tethered bees that were heated on the head was always followed by a decline, and stabilization, of T_H (Fig. 5). When the tethered bees were killed and heated again with the same heat input and with the thermocouples and heat-lamp in the same positions, T_H typically increased to 50°C , or as much as 10°C above that observed in heated live bees which stabilized their T_{Th} (Figs. 5 and 6).

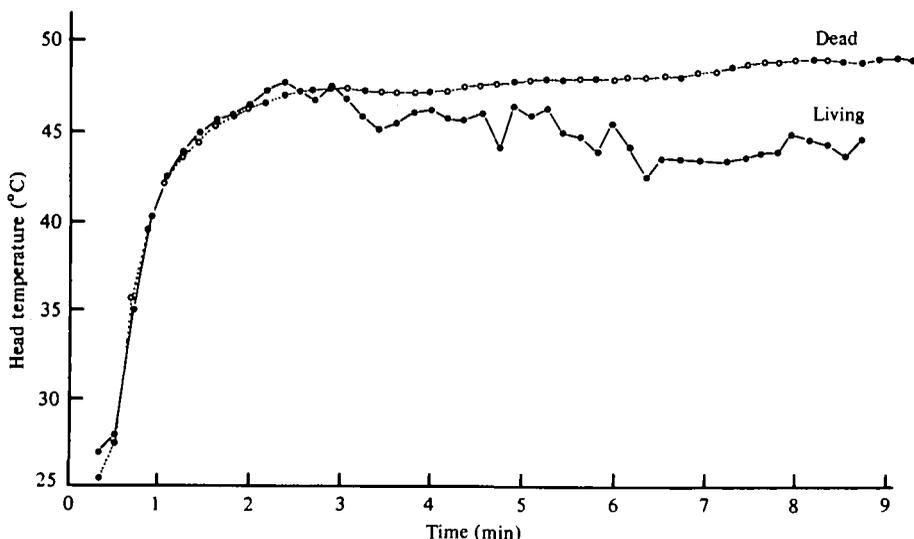


Fig. 6. A comparison between head temperature of a living bee that was extruding a fluid droplet when heated on the head and of the same bee when dead with the same heat input. $T_A = 26^\circ\text{C}$.

(D) Activity of the heart and aorta

The heart in the abdomen, and its extension the aorta in the thorax and head, pumps blood anteriorly. The aorta empties near the brain, and since it traverses the sometimes hot muscles in the thorax, the pumping activity of either the heart or the aorta could have considerable effect on head temperature.

For the most part there was relatively little apparent mechanical activity of the aorta in the head in the 27 bees examined when they were not heat-stressed. However, on occasion distinct low-amplitude pulses were evident. These pulses were generally, but not always, at the same frequency as the heart pulsations in the abdomen as well as the abdominal pumping movements. Fluid visible from pinholes punched into the top of the head revealed in-out movements in synchrony with this cycle.

A dramatic change occurred, generally within a few seconds, when the head was heated to temperatures $> 47^\circ\text{C}$. Heating of the head resulted in high-amplitude aortic pulses in the head, although there was little or no response in the activity of the heart in the abdomen (Fig. 7; Table 2). Heart pulsations in many bees decreased in amplitude at the same time that aortic pulses were increasing in frequency and amplitude.

Thoracic heating resulted sometimes in an increased pumping of the head aorta (9 instances), a decrease (4 instances), or no apparent change (8 instances). Abdominal heating, on the other hand, resulted in increased frequency of abdominal heart pulsations, but it did not change the aortic pulsations in the head (Table 2). These data suggest that the blood circulation from abdomen to thorax is, in part, independent from that between thorax and head.

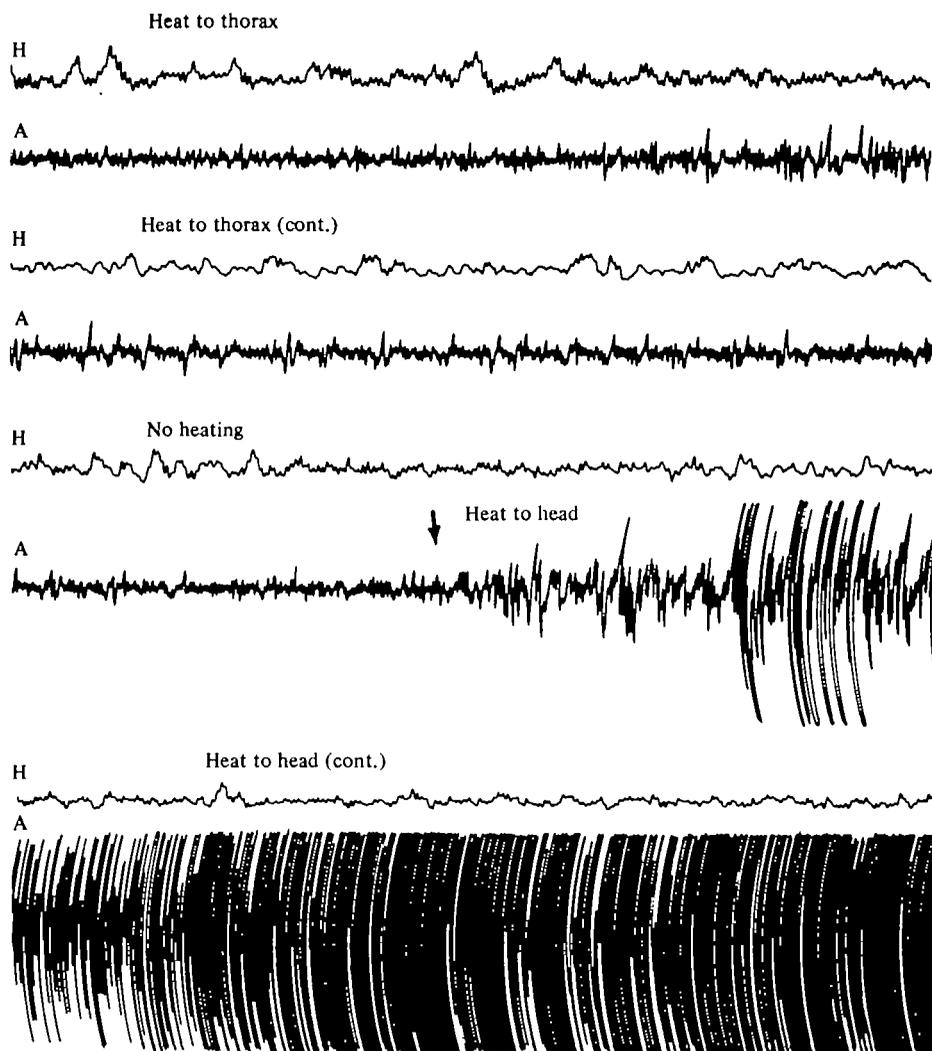


Fig. 7. Four sections of records (each spanning 46 s) of concurrently recorded aortic (A) and heart (H) activity from the same bee. The first two sections of heart (and aorta in the head) activity show mechanical responses when the thorax was heated from 33 to 44 °C. The second two sections of heart (and aorta) activity show activity during lack of external heat input, and the effect of heating of the head. Thoracic temperature declined from 46 to 44 °C (3rd section) and from 34 to 33 °C (last section). Note the reduction in abdominal heart activity during thoracic heating, and the violent aortic pulsations during heating of the head.

(E) *Temperature pulses in the head*

Sometimes after a hole was punched into the head prior to the insertion of either electrodes or thermocouples it was possible to observe in-out movement of fluid from the wound. These movements were in synchrony with abdominal respiratory movements, which were in turn in synchrony with aortic pulses (observed either with the recording equipment or visually as throbbing in the flexible membrane of the neck). These observations suggested that heating of the heart and/or the

Table 2. Effects of heating the thorax, head or abdomen to 45–50 °C on the pumping vigour (amplitude and/or frequency) of the aorta (in the head)

(The table shows number of observed occurrences in a sample of 33 honeybees.)

Part of body heated	Response in	Occurrences		
		Increase	Same	Decrease
Head	Aorta	27	0	0
	Heart	8	3	11
Thorax	Aorta	9	8	4
	Heart	6	10	8
Abdomen	Aorta	0	10	3
	Heart	10	2	0

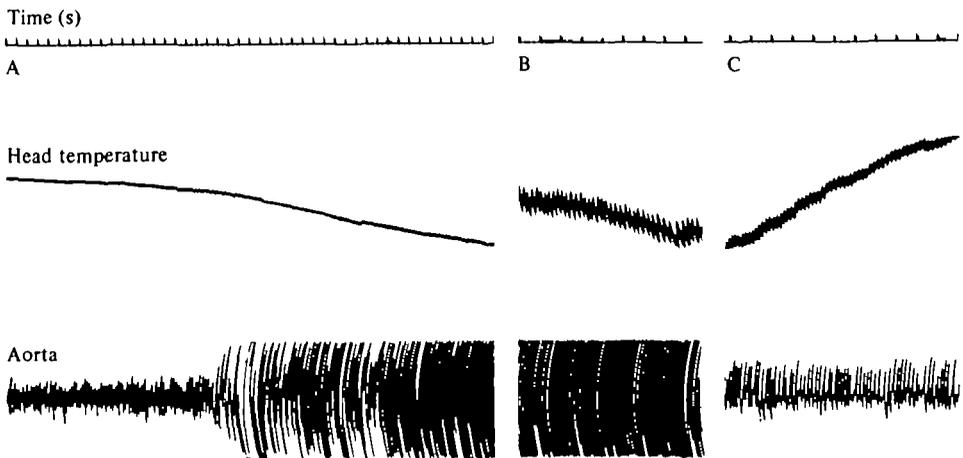


Fig. 8. Relationship between activity of the aorta in the head and temperature pulses in the head in the same bee. The largest temperature pulses (in B) are approximately 0.1 °C. Downward inflexion of the head temperature on this recording indicates an increase in T_H . (A) Heating of head from 38 to 48 °C, $T_{Th} = 33-36$ °C. (B) stabilization of T_H at 45 °C, $T_{Th} = 36$ °C. (C) Head cooling. $T_H > 40$ °C. $T_{Th} = 33$ °C.

aortic, or abdominal pumping movements, functioned to move blood and possibly heat between thorax and head.

During head heating the aortic pulses were often independent of the heart pulsations, and the heart pulsations were, in turn, sometimes independent of the abdominal (respiratory) pumping movements. Which of the three pumping rhythms are primarily responsible for the heat transfer between head and thorax?

Highly amplified signals from thermocouples implanted in the head indicated that the heart activity was often matched, beat by beat, with temperature changes in the head, although independent of aortic pulsations and abdominal pumping movements (Figs. 8 and 9). When the head was heated these temperature changes (of the order of 0.001 °C) registered by the 46-gauge thermocouple indicated an influx of a pulse of medium (presumably blood) at lower temperature.

It is possible that the aortic pulses and the abdominal pumping modify or amplify

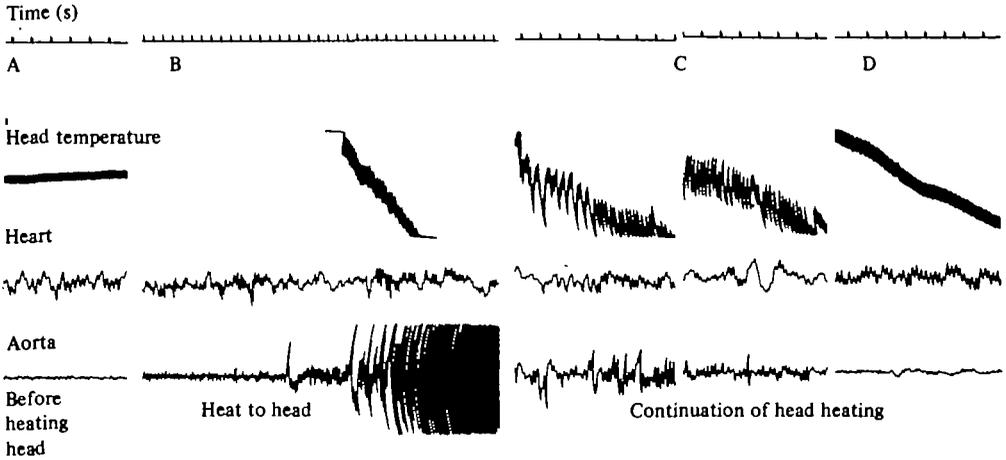


Fig. 9. Interrelationships between activity of the heart in the abdomen (centre), the aorta in the head (bottom), and temperature pulses in the head (top) in the same bee. Seconds are indicated in top margin of the records. (A) Before head heating, $T_{Th} = 28^\circ\text{C}$, $T_H = 25^\circ\text{C}$. (B) Application of heat to the head till $T_H > 44^\circ\text{C}$. (C) and (D) Continuation of head heating as T_H stabilizes near 49°C while the bee is extruding a droplet. $T_{Th} = 37\text{--}38^\circ\text{C}$. Temperature pulses are in synchrony with abdominal heart pulsations. In D the activity of the aorta in the head has been obliterated by heating the head $> 50^\circ\text{C}$. $T_H = 50^\circ\text{C}$, $T_{Th} = 36^\circ\text{C}$. The record shows that temperature pulses in the head cease even though abdominal heart activity may continue. Downward inflexion of T_H in this record indicates increase of T_H . The largest temperature pulses (in C) are approximately 0.2°C .

the heat transfer response of the heart, but the present methods were not sensitive enough to detect this directly. However, indirect observations indicate it. For example, vigorous heart pulsations do not necessarily lead to temperature pulsations in the head. When the activity of the aorta is selectively obliterated (by overheating the head to near lethal temperatures) without affecting the abdominal heart, this results in the cessation of temperature pulses in the head (Fig. 9).

DISCUSSION

The present data indicate that honeybees regulate head temperature, but they do so apparently using only one upper set-point. They prevent overheating of the head at high T_A by evaporative cooling using regurgitated honeycrop contents (Fig. 10). Heat is redistributed by conduction, by in-out movements of the fluid droplet, and by aortal pulsations which presumably stir the blood. The temperature sensor for this response appears to be located in the head itself, since heating of the thorax to near lethal temperatures generally elicits neither the regurgitation nor the associated response of the aorta, unless T_H also becomes elevated above at least 45°C .

At low T_A head temperature is not independently regulated. Instead, T_H at low T_A is primarily a function of T_{Th} . Head temperature becomes elevated primarily due to passive conduction of heat from the thorax, and head temperature is thus 'regulated' only to the extent that T_{Th} is regulated. Active regulation of T_{Th} in honeybees has been well studied. At low T_A the bees warm up, shivering as needed (Bastian & Esch, 1970), to maintain T_{Th} near or above 30°C (Esch, 1960).

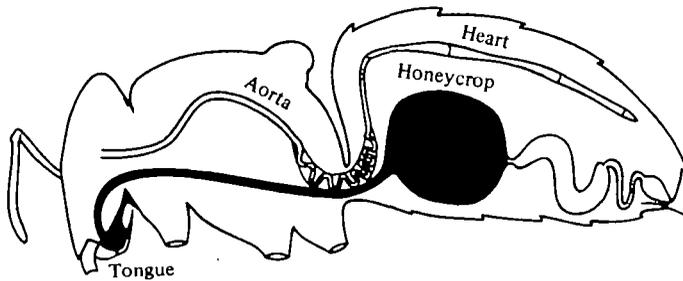


Fig. 10. Diagrammatic representation of a honeybee, showing the honeycrop and regurgitated crop contents held on the tongue, and the circulating system with the convoluted aorta in the petiole.

The low-temperature sensor(s) may reside in the thorax itself, rather than in the head, the apparent site of the high-temperature sensor in honeybees. Heating of the thoracic muscles necessarily also heats the ganglia in the thorax. At least in the moth, *Hyalophora cecropia*, the temperature of the thoracic ganglia determines whether or not the animals engage in warm-up or flight behaviour (Hanegan, 1973). Temperature of the thorax also affects thermoregulatory behaviour of other insects. In the sphinx moths, *Manduca sexta* (Heinrich, 1971), and in the bumblebee, *Bombus vosnesenskii* (Heinrich, 1976), overheating of the thorax stimulates the heart in the abdomen to pump cool blood through the thorax, thus causing T_{Th} to decline.

In the honeybees thoracic heating by itself did not greatly stimulate heart activity, but pulses of low temperature recorded in the head were in synchrony with heart pulsations and not necessarily in synchrony with pulsations of the aorta in the head. These data demonstrate that the activity of the aorta can be independent of the activity of the heart. This suggests further that the blood circulation between abdomen and thorax may be, in part, independent of the circulation between thorax and head. Possibly the aortic pulsations modify blood flow to the head that is driven by the abdominal pump. However, the present data do not allow one to distinguish among several alternate mechanisms of fluid-flow mechanisms.

It is of interest that the regurgitation of fluid has several functions. First, it is used to empty the honeycrop to feed hivemates (in honeybees) and larvae, and to fill empty storage cells. Secondly (in bumblebees), it is used by young queens (at both low and high T_A) prior to hibernation apparently to concentrate nectar to ensure a high calorific content of the honeycrop (Heinrich, unpublished). Lindauer (1954) reported the behaviour for honeybees in an overheated hive and studied it relative to hive temperature regulation. Esch (1976) first reported the behaviour in bees flying at high T_A and speculated that it might be used for the control of body temperature of individual bees. The present study has confirmed and extended these observations and speculations.

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