ley, O. J. Balchum, *Arch. Environ. Health* **16**, 648 (1968); B. D. Goldstein, O. J. Balchum, H. B. Demopoulos, P. S. Duke, *ibid.* **17**, 46 (1968); B. D. Goldstein, C. Lodi, C. Collinson, O. J. Balchum, *ibid.* **18**, 631 (1969).
3. Nutritional Biochemicals Corp., Cleveland; H. H. Draper *et al.*, *J. Nutr.* **84**, 395 (1964).
4. L. Friedman, W. Weiss, F. Wherry, O. L. Kline, *J. Nutr.* **65**, 143 (1958).
5. General Biochemical Corp., Chagrin Falls, Ohio.
6. M. L. Quaife, N. S. Scrimshaw, O. H. Lowry, *J. Biol. Chem.* **180**, 1229 (1949).
7. Student's *t*-test.
8. C. E. Mengel and H. E. Kann, *J. Clin. Invest.* **45**, 1150 (1966); L. A. Witting, *Lipids* **2**, 109 (1966); L. J. Machlin, *J. Amer. Oil Chem. Soc.* **40**, 368 (1963).
9. M. P. Carpenter, A. E. Kitabchi, P. B. McCay, R. Caputto, *J. Biol. Chem.* **234**, 2814 (1959); A. E. Kitabchi and R. H. Williams, *ibid.* **243**, 3428 (1968); S. T. Pritchard and H. Singh, *Biochem. Biophys. Res. Commun.* **2**, 184 (1960); J. G. Bieri and A. A. Anderson, *Arch. Biochem. Biophys.* **90**, 105 (1960).
10. H. V. Thomas, P. K. Mueller, R. L. Lyman, *Science* **159**, 532 (1968).
11. D. Harman, *J. Gerontol.* **12**, 199 (1957); A. L. Tappel, *Fed. Proc.* **24**, 73 (1965).
12. L. D. Scheel, O. J. Dobrogorski, J. T. Mountain, J. L. Svirbely, H. E. Stokinger, *J. Appl. Physiol.* **14**, 67 (1959).
13. We thank Dr. C. Loosli for guidance and assistance and R. Cuzzi-Spada for his technical aid. Supported by NIH grant No. ES 00430 and by the Hastings Foundation.

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Nervous Control of the Heart during Thoracic Temperature Regulation in a Sphinx Moth

Abstract. *Heating the thorax of the sphinx moth,* Manduca sexta, *evoked pulsations of the heart in the abdomen. These pulsations were of relatively high rate and amplitude, and traveled from the abdomen into the thorax. While heat was continuously applied exclusively to the thorax, thoracic temperature often stabilized and abdominal temperature increased sharply. Thoracic heating of moths with transected nerve cord, however, did not evoke these responses. It is inferred that the heart in the abdomen responds to overheating of the thorax through neural influence.*

Descriptive knowledge of the insect circulatory system dates back to Harvey (*1*) and has recently been reviewed (*2, 3*). This circulatory system is an open one, although blood is pumped by a pulsatile vessel, the heart. In some insects, the heart is segmentally innervated from the ventral nerve cord (*4*), but that of saturniid moths, for example, is thought to be exclusively myogenic (*5*).

The heart is a muscular tube extending along the middorsal line of the abdomen into the thorax (Fig. 1). The so-called alary muscles are laterally attached. Segmentally arranged pairs of ostia allow inflow of blood, and ostial valves prevent outflow (*6, 7*).

In the sphinx moth, *Manduca sexta*, blood circulation is involved in the stablization of thoracic temperature during free flight over a wide range of ambient temperatures (*8*). It was not known if thoracic temperature was regulated, or if stabilization was achieved automatically by the pumping of the heart in the abdomen at its own temperature-dependent rate. This rate might be rapid when the abdomen is hot and slow when the abdomen is cool, as it is at low ambient temperature. I now report, however, that the pumping of the heart in the abdomen is modulated in response to thoracic temperature.

The moths used in this study were supported by their wings from a frame of balsa wood so that their bodies were freely suspended. Heat was directed onto the thorax with a narrow beam of light from an incandescent lamp. During thoracic heating, both the heart pulsations and the body temperatures were simultaneously and continuously recorded. The latter were measured with 40-gauge copper-constantan thermocouples implanted in the thorax, as well as in the second and fifth abdominal segments (Fig. 1). The leads from the thermocouples were connected to a multichannel potentiometric recorder.

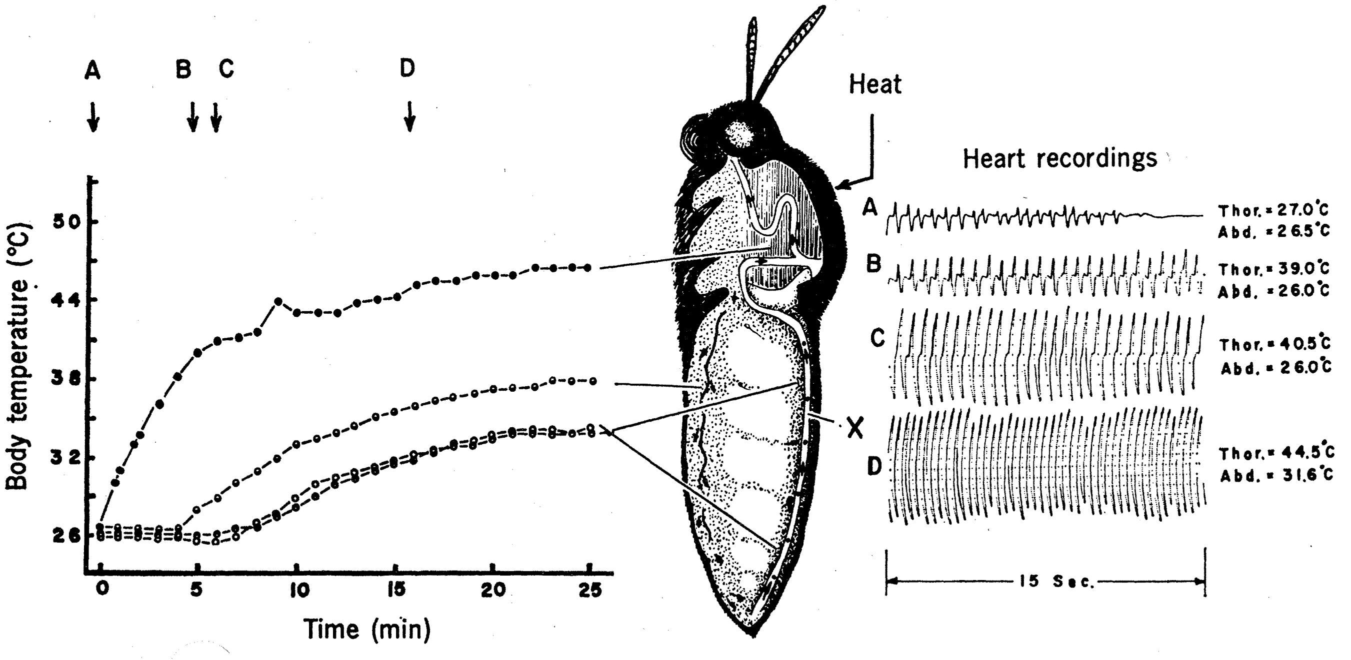

Fig. 1. Body temperatures and abdominal heart recordings from the same run during the application of heat exclusively to the thorax of a moth. Heating of the thorax was initiated at minute 0. Lines on the sagittal view of thorax and abdomen point from the positions of the implanted thermocouples to the respective temperatures recorded from that area during the 25 minutes of heat application to the thorax. The approximate position of the electrodes, which were implanted laterally along the abdominal heart to record heart pulsations, is indicated by X. The specific times at which the abdominal heart pulsations were recorded are indicated by *A*, *B*, *C*, and *D*. The same moth, when dead, was again heated in a similar manner. The initial rate of temperature increase in the thorax was the same as in the live moth; however, after 12 minutes of thoracic heating, the dead moth had a thoracic temperature of 53°C; abdominal temperature did not exceed 26°C during this time.

The pulsations of the heart were monitored simultaneously in the thorax and in the abdomen by silver electrodes implanted on each side of the heart. The electrodes were wired to a pair of Biocom impedance converters, the signals of which were recorded on separate channels of a polygraph. The movements of the heart, observed through the transparent integument, coincided with the polygraph records. The impedance measurements recorded the timing of the heart pulsations as well as their relative amplitude.

The frequency, amplitude, and duration of continuous pulsations of the heart increased when the thorax was heated to 40° to 43°C, even though the abdominal temperature initially did not change and remained relatively low (Fig. 1). In moths with heated thoraxes, the pulses usually moved from the tip of the abdomen into the thorax. These pulses were always at the same frequency in the thorax and in the abdomen, even though the temperatures of thorax and abdomen were made to differ by more than 20°C. Individual pulses in the abdomen were usually recorded a few milliseconds before those in the thorax.

It can be inferred from the heart pulsations (Fig. 1) that the flow of blood through the heated thorax was relatively strong. When the heat input to the thorax was not excessive, the temperature of the thorax often stabilized. This stabilized thoracic temperature was always lower than that observed during thoracic heating of dead moths. Since the thorax of the moth shown in Fig. 1 was deliberately overheated, the rate of heat transfer from thorax to abdomen was probably maximum; the temperature of the second abdominal segment rose up to 8°C in the dorsum and 11°C in the ventrum (Fig. 1). However, when the ventral nerve cord was transected (posterior to the second abdominal segment) and the thorax was heated as before, the increase in abdominal temperature was only 2° to 3°C, as in dead moths.

Transection of the nerve cord immediately reduced the frequency of the heart pulsations. In operated moths, in contrast to normal ones, heating of the thorax did not evoke either increased rates or amplitudes of pulsation of the heart in the thorax or in the abdomen. Unlike the situation observed in unoperated moths with high thoracic temperatures, the pulses of the heart in operated moths sometimes moved from thorax to abdomen.

Four out of seventeen unoperated moths failed to show increases of heart rate, pulse amplitude, abdominal temperature, or stabilization of thoracic temperature in response to thoracic heating. It is inferred, in these cases, that the ventral nerve cord, or nerves innervating the heart or its alary muscles were damaged by one of the many implanted thermocouples or electrodes.

A number of anatomical features of sphinx moths contribute to temperature regulation. The thick layer of thoracic scales (*9*) and the hollow space (*7*) between thorax and abdomen (Fig. 1) aid in the retention of heat in the thorax, and should therefore be useful whenever the moths are flying in low (<15°C) air temperatures, when blood flow from the abdomen to the thorax appears to be nearly cut off (*8*).

The blood from the thorax flows under and around the ventral diaphragm. This membrane, extending along the midventral line of the abdomen, undulates laterally and posteriorly (*3*, *6*). It aids in stirring and propelling the blood which collects in the heart after passing through the hemocoel, where heat is lost by convection and evaporation (*9*, *10*). The blood appears to have cooled maximally by the time it has entered the heart (Fig. 1). It is then impelled into the thorax where the pulsatile vessel loops through the flight musculature (*7*, *11*). This loop should act as a cooling coil.

It can be concluded that the pumping of the heart is affected by impulses from the thorax via the ventral nerve cord. This affords a mechanism for control of the movement of heated blood from the well-insulated thorax into the poorly insulated abdomen, and allows the abdomen to serve as a radiator where heat from the thorax is unloaded via the blood.

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References and Notes

1. W. Harvey, *Exercitatio Anatomica de Moto Cordis et Sanguinis in Animalibus* (Sumptibus Guilielimi Fitzeri, Francofurti, 1628).
2. J. C. Jones, in *The Physiology of Insecta*, M. Rockstein, Ed. (Academic Press, New York, 1964), chap. 1.
3. A. G. Richards, *J. Morphol.* **113**, 17 (1963).
4. N. E. McIndoo, *J. Comp. Neurol.* **83**, 141 (1945).
5. F. V. McCann, *Ann. N.Y. Acad. Sci.* **127**, 84 (1965).
6. J. H. Gerould, *Acta Zool. (Stockholm)* **19**, 297 (1938).
7. F. Brocher, *Arch. Zool. Exp. Gen.* **58**, 149 (1919).
8. B. Heinrich, *Science* **168**, 580 (1970).
9. N. S. Church, *J. Exp. Biol.* **37**, 172 (1960).
10. H. Esch, *Z. Vergl. Physiol.* **43**, 305 (1960); P. A. Adams and J. E. Heath, *J. Res. Lepidoptera* **3**, 69 (1964).
11. E. Burgess, *Proc. Boston Soc. Natur. Hist.* **21**, 153 (1881).
12. This work was carried out as part of a doctoral research project. It was supported by grant GB 18744 X to Dr. G. A. Bartholomew to whom I am grateful. I also thank Dr. F. Englemann for reading the typescript.

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Junctional Membrane Permeability: Restoration by Repolarizing Current

Abstract. *In cells of the* Chironomus *salivary gland, junctional membrane conductance, depressed by various chemical treatments, is restored to its normal high level by currents passed inward through nonjunctional cell membrane.*

The high membrane permeability at the junction of a variety of cells is depressed by cell injury (*1–3*), by intracellular injection of Ca^{2+} (*3*), by anisotonic media (*3*) or media free of Ca and Mg (*4*), by media in which propionate substitutes for Cl^- (*2*) or Li^+ substitutes for Na^+ (*4*), by low temperature (*5*), by chemical metabolic inhibitors (*6*), and by electric current going outward through the nonjunctional membrane (*7*). Under most, if not all, of these conditions depolarization takes place at various rates and to various degrees. Thus the question arose whether repolarizing (inward-going) current, applied at times of low junctional permeability (uncoupled state), will restore high junctional permeability. I have examined this question for three of the aforementioned conditions of uncoupling and found that restoration indeed takes place.

Salivary glands of mid-fourth instar larvae of *Chironomus thummi* were isolated in a physiological solution [normal medium (*8*)] and then exposed continuously to one of the following uncoupling media: dinitrophenol (DNP) medium, medium free of Ca and Mg, and medium free of Ca and Mg but with Li (*8*). Two adjacent cells I and II (Fig. 1) were impaled, each with one voltage-recording (E_I, E_{II}; filled with KCl) and one current-recording microelectrode [i_I, i_{II}; filled with KCl,