control systems; although, no honest or immediate attempt will be made to explain this unusual phenomenon.

There was no noticeable difference in the timing of the onset of the body temperature rhythm between December and February controls; although differences in the amount of activity and/or arousal were believed to be partly associated with the longer time that February bats spent in hibernation prior to laboratory cold exposure as compared to December bats. Members of both control groups appeared healthy and in good physiological states prior to and during experimental cold exposure. The similarity in the timing of the onset of the body temperature rhythm is new evidence supporting the existence of a biological rhythm in hibernating bats at low ambient temperatures.

Winter bats when exposed to the cold revealed an endogenous-type 24-h body temperature rhythm or a multiple of the 24 h rhythm regardless of the number of days (0–48) they had been held at 33°C prior to cold exposure. These rhythms were either in the form of a major rise in body temperature to the active level or a more subtle rise of one or two degrees. The rise in body temperature during the laboratory cold exposure in total darkness occurred quite regularly between the hours of 15.30 and 18.30 (averages appear in the Figure). The onset of arousal coincided with the onset of winter darkness; although bats had been exposed to a summer-like day-night cycle at 33°C prior to cold exposure. This phenomenon suggests that biological rhythms in species of hibernating bats, such as the onset of body temperature cycling for *M. lucifugus*, are established in nature from environmental cues and are maintained for a period of time even under a new or changing regime of envir environmental conditions in the laboratory.

If changes in arousability and activity are indicesindices of heat acclimation, we can conclude that bats in wirin winter probably become heat-acclimated between 2–4 we–4 weeks when held at a neutral temperature of 33°C, although hough heat acclimation has little noticeable effect on the onset of set of the body temperature rhythm. This period of time for ie for the establishment of heat acclimation is consistent with twith that reported for true homeotherms[6]. Heat-acclimated wired winter bats arouse from the cold in a manner similar to thato that reported for true summer bats[11].

Zusammenfassung. Kleine braune Fledermäuse, *Myse*, *Myotis lucifugus*, wurden dem Winterschlaf entzogen und eiund einer neutralen Temperatur von 33°C während 0–48 Tagen Tagen ausgesetzt und darauf drei Tage lang 10°C. Bei Fledermäutermäusen, welche Kälte (10°C) ausgesetzt waren, erwies die Ti die Temperaturkurve im allgemeinen, dass das Verhältnis ältnis und das Ausmass des Erwachens sowie die Regelung ;elung der Tätigkeit oder Körpertemperatur proportional mit il mit der Dauer abnahmen, während welcher sie der Hitze (33tze (33°C) ausgesetzt waren.

R. C. Stones[12] and J. E. Wieb. Wiebers

Department of Biological Sciences, Purdue University,versity, Lafayette (Indiana USA), June 15, 1964.

[12] Present address: Department of Biological Sciences, Miches, Michigan Technological University, Houghton (Michigan USA).

Daily Rhythms in the Endocrine Glands of Drosophila Larvae

Many important functions of insect larvae, such as metabolism, growth, molting, and differentiation, are under the control of hormones, which are produced in the neurosecretory cells, the corpora allata, and the prothoracic glands[1]. At the same time some of these functions are controlled by circadian rhythms, as for example the timing of developmental steps like the pupal molt[2] or eclosion[3] in Drosophila. Therefore, it seemed of major interest whether the endocrine system in Drosophila larvae is connected somehow with the 'clock', as studies on adult flies have suggested[4]. In Dipteran larvae the corpus allatum is located in the dorsal, and the prothoracic gland in the lateral part of the ring gland; most neurosecretory cells are found in the pars intercerebralis of the brain[5]. An indication of the secretory activity of gland cells can be derived from the size of their nuclei and nucleoli[6], which may be proportional to the amount of RNA synthesis[7].

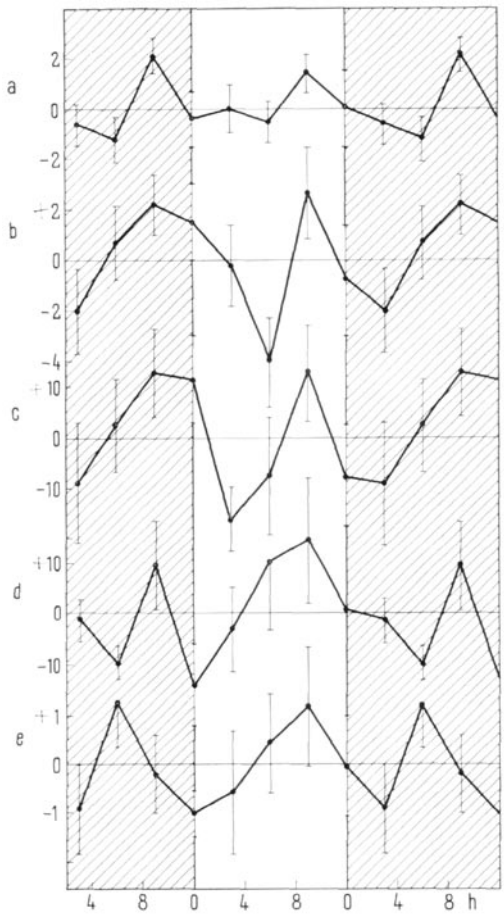
Larvae from highly inbred stocks of *Drosophila melanogaster* (Meig.) were raised synchronously under standardized conditions. They were kept at a controlled constant temperature of 20°C in two cabinets with a 12 h light 12 h dark cycle, one with the light period from 10.00 to 22.00, the other from 22.00 to 10.00. At the desired age larvae from both cabinets were killed at 3 h intervals over a period of 12 h, and then immersed in Carnoys or Bouins fixative. Sections of 4 μ were cut and stained for RNA[8] or neurosecretory material[9]. The methods of mds of measuring the size of the nuclei and nucleoli were the samhe same as described earlier[4]: the values for the long and short dishort diameter of every nucleus were multiplied and the prohe product taken as an indication of nuclear size. The diameteiameters of the nuclei measured between 3 μ and 20 μ, and the pi the precision of the measurements was ± 0.3 μ. Ten or m or more nuclei of each tissue of every larva were measured asured and individual means determined; about ten individual mdual means were averaged and standard errors calculated for ei for every point in the curve for the 6th day of development pment (one day before puparium formation).

Since the larvae are growing, any possible fluctuafluctuation of nuclear size must be determined relative to the ov the overall growth trend. We measured and calculated the grohe growth, which in larval tissue takes place without cell divisll divisions, for each tissue from the 4th to the 7th day of larval larval life.

[1] H. A. Schneiderman and L. I. Gilbert, Science *143*, 325 (13, 325 (1964).
[2] L. Rensing, unpublished.
[3] C. S. Pittendrigh, Proc. Nat. Acad. Sci. U.S. *40*, 1018 (19.018 (1954).
[4] L. Rensing, Science *144*, 1586 (1964).
[5] H. Köpf, Zool. Anz., Suppl. *21*, 439 (1957).
[6] T. O. Casperson, *Cell Growth and Cell Function* (W. W. N. W. Norton, New York 1950).
[7] J. J. Taylor and P. S. Woods, in *Subcellular Particles'articles* (Ed.: T. Hayashi; Ronald Press, New York 1959).
[8] M. H. Flax and M. Himes, Physiol. Zool. *25*, 297 (1952). 1952).
[9] M. Gabe, Bull. Microscop. appl. *3*, 153 (1953).

From every point of the daily curve the corresponding value of the growth curve was subtracted and the plus or minus deviations plotted. The variability of the nuclear size between individuals in a group was in the range of 12–22% in the corpus allatum and fat body cells, and 25–31% in the prothoracic gland cells; multiple measurements on one individual indicated an average sampling error of 6.5%.

The Figure a–d shows the changes in the size of the nucleus of neurosecretory cells, corpus allatum, fat body and prothoracic gland cells during 24 h; all show a bimodal pattern with maxima consistently occurring 3 h before dawn and dusk. The maxima and minima of neurosecretory, corpus allatum and fat body cells are significantly different in their values as measured by a t-test ($p = 0.01$–0.05). The neurosecretory cells (Figure a) display a higher peak before dawn compared to that before dusk, but no obvious differences in the shape of these two maxima. Corpus allatum and fat body nuclei (Figure b, c) show a very similar pattern, except that the peak at dawn is somewhat broader than that at dusk. The nuclei of the prothoracic glands (Figure d) vary considerably in their size, but again two peaks appear, the broader one this time before dusk and the smaller and steeper before dawn. The nucleoli of the prothoracic glands (Figure e) exhibit a similar pattern of size change, except that one peak occurs 3 h earlier.



Daily fluctuations in nuclear (a–d) and nucleolar (e) size relative to growth. a, Neurosecretory cells; b, corpus allatum cells; c, fat body cells; d, e, prothoracic gland cells. Abscissa – time in h after dawn and dusk; the night part of the Figure is repeated. Ordinate – deviations from the average growth as described in the text. Standard errors indicated by the vertical lines.

No rhythm was expected in the fat body cells which were included as a control. The fact that the fat body cells also displayed a rhythm similar to the other glands led us to check the overall size of some samples, but neither the size of the brain nor the size of the whole section seemed to follow a comparable pattern. Other possible explanations of the findings, for example synchronous mitosis or endomitosis or uneven treatment of the slides, could not be found, and it is concluded that the observed variations in nuclear size most likely reflect the metabolic activity of the cell. The fat body, which is involved in fat and protein storage and metabolism and which has perhaps an analogous function to the vertebrate liver[10], may very well change its activity rhythmically, controlled by corpus allatum[11] and prothoracic gland hormone[12].

For the first time a circadian rhythm is indicated for the ecdysone production, suggesting that other ecdysone-dependent phenomena may also change rhythmically. Neurosecretory cells and corpus allatum exhibit a pattern very similar to that found in adult flies[4]; the corpus allatum, however, seems to have a different phase angle relative to the neurosecretory cells: in adults corresponding points on the curve occur 3 h earlier, in larvae either at the same time or 12 h apart. The results in general underline the importance of hormones in daily rhythmic phenomena like molting, pupation, emergence[13], locomotory activity and metabolism[14].

Zusammenfassung. Neurosekretorische Zellen, Corpus allatum, Prothoraxdrüsen- und Fettkörperzellen in Drosophilalarven zeigen tägliche, zweigipflige Schwankungen der Zellkerngrösse. Ein ähnliches Muster konnte auch in den Nucleoli der Prothoraxdrüsen beobachtet werden.

L. RENSING[15], B. THACH, and V. BRUCE

Biology Department, Princeton University, Princeton (New Jersey USA), September 23, 1964.

[10] S. C. MUNSON, in *Insect Physiology* (Ed.: K. D. ROEDER; John Wiley & Sons, New York 1953).
[11] E. THOMSEN, J. exp. Biol. *29*, 137 (1952).
[12] V. B. WIGGLESWORTH, Symp. Soc. exp. Biol. *11*, 204 (1957).
[13] L. RENSING, in preparation.
[14] These experiments were conducted in Professor PITTENDRIGH's laboratory and in part represent the Senior Thesis work of the second author. We gratefully acknowledge support of grant No. 1858(28) from the Office of Naval Research.
[15] Present address: 1. Zoologisches Institut, Göttingen (Germany).