arousal from hibernation, glutamine and glutamic acid content remained practically unaltered. Concentrations of GABA, however, exhibited a small but steady and significant tendency to decrease as the animal approached full arousal from the hibernating state ($p > 0.01$). Concentrations of the investigated amino acids, estimated in the brain of animals artificially refrigerated to 5–7°C, were also significantly lower than the corresponding values found in the brain of euthermic animals. As is seen in the Figure, their decrease was of approximately the same order as in the hibernating animals sacrificed at the same rectal temperature. In contradistinction to the arousal from hibernation, the process of warming from hypothermia was characterized by a highly significant increment ($p > 0.001$) of glutamine in the brain. The sharpest increase of this amino acid, observed between 5 and 15°C, was accompanied by an important decrease of cerebral glutamic acid. As the warming process proceeded, concentrations of both compounds increased and, with restitution of euthermia, attained the control values. The increase in the concentrations of GABA, although less noticeable, proved to be statistically highly significant ($p > 0.001$) once the animal reached the temperature of 35–37°C.

The results described call for a few brief comments. Unless it is believed that the differences observed in our experiments are due to the use of the specific method of cooling, one is tempted to infer that the glutamate-GABA metabolism in the brain of ground squirrels waking from hibernation, and those spontaneously warming up from an artificially induced state of hypothermia, follows two different pathways. Experiments using other methods of inducing hypothermia are at present being carried out in order to check this assumption. The significant tendency of GABA to decrease as the animal wakes up from the hibernating state also merits special attention. It provides another (among many) reasons for not rejecting the possibility that GABA might indeed play an important role in the regulation of cerebral excitability[3].

Résumé. Chez le spermophile, la teneur en glutamine et en acide glutamique cérébral au cours du réveil du sommeil hivernal était inchangée. Pendant le réchauffement des animaux refroidis, la glutamine et GABA étaient remarquablement élevés.

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Body Temperature Cycling of Winter Little Brown Bats in the Cold Following Heat Exposure[1]

Heat and cold acclimation studies have been conducted for a number of homeotherms, including white rats[2,3], brown rats[4], and rabbits[5]. In general, homeotherms which have been heat-acclimated for 3–5 weeks reveal lower metabolic rates and poorer body temperature regulation at low ambient temperatures when compared to cold-acclimated homeotherms[6]. Hart further states that acclimation in homeotherms is developed in about 2–4 weeks under laboratory conditions, and that the primary function of cold acclimation is to extend the survival range for a limited period of time.

Little is known about acclimation in heterotherms with the exception of spermophiles[7] and dormice[8]. These two mammals and acclimatized (natural climatic exposure) bats[9], have shown a body temperature metabolism pattern similar to true homeotherms. The aim of the present investigation was to determine the effect of progressive heat exposure on body temperature cycling and arousal patterns for winter-captured little brown bats, *Myotis lucifugus*, in the cold. An attempt was made to determine if changes in body temperature could be used as an index for heat acclimation in hibernating species of bats.

Methods. Equal numbers of male and female little brown bats were captured from a Southern Indiana cave in December 1962. The bats were transferred to the laboratory, individually housed at a neutral temperature of 33°C (heat exposure) and fed daily on adult mealworms. A day-night cycle of 14 h of light (05.00 to 19.00) and 10 h of dark (19.00 to 05.00) accompanied the heat exposure. Relative humidity was 18–20%.

At four-day intervals (4–48 days) a group of two males and two females was taken from the heat (33°C) and exposed for three days (72 h) to the cold (10°C) in total darkness. 10°C was selected as the cold-test temperature as it approximates the upper limit of most winter cave hibernaculae[10], but still permits winter hypothermic bats to arouse and raise their body temperature to the homeothermic level[11].

Body temperature of the bats was measured using 30-gauge copper-constantan thermocouples implanted subcutaneously in the upper mid-abdominal region in conjunction with an automatic, strip chart recording potentiometer.

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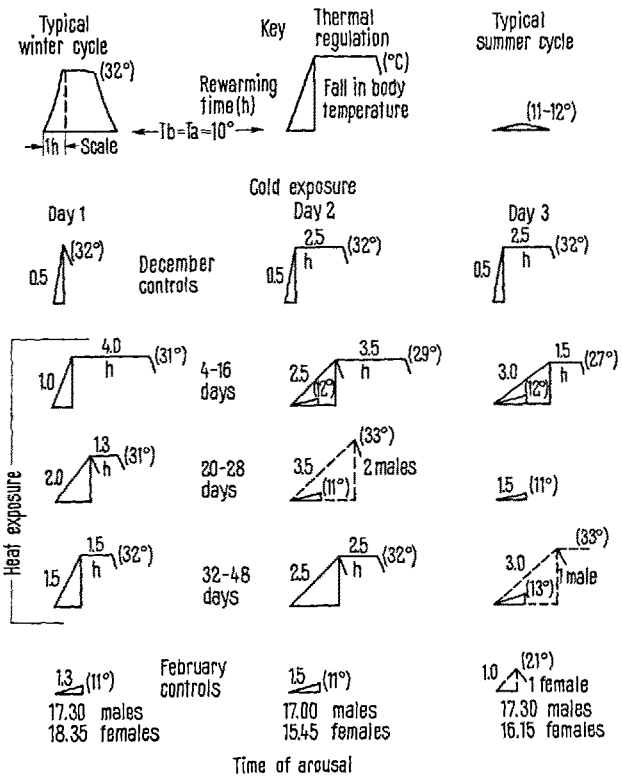
Results. The effects of prior heat exposure (0–48 days at a neutral temperature of 33°C) on the body temperature cycling pattern of bats in the cold (3 days at 10°C) have been graphically portrayed in the Figure. A summary of the changes in the rate and height of arousal, rhythmicity and activity or body temperature regulation are discussed below for groups of control and heat-exposed bats.

(1) *December controls* (zero days at 33°C). These bats were removed from a cave in December and immediately exposed to the laboratory cold for three days. They periodically aroused from 10°C and consistently rewarmed to homeothermic levels of body temperature (32°C) in 0.5 h. The rate and height of bat arousals were nearly identical during each of the three days. Once the bats had attained high levels of temperature on the second and third days, homeothermy was maintained for approximately 2.5 h before they again became hypothermic.

(2) *Heat-exposed bats* (4–16 days at 33°C). These bats were removed from a cave at the same time as the above controls and exposed to laboratory heat for 4–16 days prior to a three-day cold exposure. This group showed some signs of heat acclimation or reduced arousability in the cold; that is, it took the bats longer to rewarm on any of the three days as compared to the controls. The longer these bats were in the cold the longer it took them to rewarm (1.0 h during the first day, 2.5 h during the second day, 3.0 h during the third day). Also, the maximum homeothermic level attained during each arousal steadily decreased from 31°C during the first day to 27°C on the third day. After reaching this high temperature the ability to hold it constant decreased from 4.0 h on the first day to 1.5 h on the third day. A few bats on days 2 and 3 showed a thermogenic rise in body temperature of only a few degrees.



Body temperature cycling patterns for little brown bats, *Myotis lucifugus*, during a three-day cold exposure (10°C) following 0–48 days' heat exposure at a neutral temperature (33°C). During heat exposure all animals had a summer-like day-night cycle of 14 h of light (05.00 to 19.00) and 10 h of dark (19.00 to 05.00), while during cold exposure it was continually dark.

(3) *Heat-exposed bats* (20–28 days at 33°F). These bats were removed from a cave at the same time as the above controls and exposed to laboratory heat for 20–28 days prior to a three-day cold exposure. They experienced the greatest reduction of body temperature regulation or the ability to arouse from the cold as compared to other bats of this experiment. On the second and third days the body temperature rose only one degree above the ambient, with the exception of two males on day 2 which warmed to 33°C in 3.5 h.

(4) *Heat-exposed bats* (32–48 days at 33°F). These bats were removed from a cave at the same time as the above controls and exposed to laboratory heat for 32–48 days prior to a three-day cold exposure. This group, instead of revealing a presupposed greater reduction of body temperature regulation than bats exposed 20–28 days, showed signs of a partial reversal in the heat acclimation pattern. They aroused faster and maintained homeothermy longer than bats exposed to heat 20–28 days. However, the overall pattern of body temperature cycling is similar to other heat-exposed groups, particularly the arousal time. Bats aroused faster to homeothermic or active levels on the first day (1.5 h) than on the second (2.5 h) or third days (3.0 h).

(5) *February controls* (zero days at 33°C). These bats were removed in February from the same cave as the above bats and immediately exposed to the laboratory cold (10°C) for three days in a manner similar to the December controls. These bats did not respond like the earlier controls. There was little activity or major change in body temperature during cold exposure. Bats failed to raise their body temperature more than one degree with the exception of one female on the third day. Bats of this control group remained torpid throughout the three-day cold exposure. Artificial stimulation at the end of the experiment caused three of four bats to arouse rapidly to active levels with no apparent difficulty.

The timing of the onset of PM arousals in body temperature did not appear to vary significantly between bats of the two control groups or among the heat-exposed groups; however, there appeared to be considerable variation by sex. The average male arousal was 17.30 on the first and third days and 17.00 on the second day. On the other hand, females averaged 18.35 (day 1), 15.45 (day 2) and 16.15 (day 3).

Discussion. When bats were removed from a natural cave hibernacula in December and immediately exposed to 10°C in the laboratory, there was no apparent difficulty in arousal from hypothermia to active or homeothermic levels. On the other hand, when these bats were exposed to heat, following their removal from a winter cave hibernacula, the body temperature pattern for arousability abruptly changed. Bats exposed 4–28 days at a neutral temperature of 33°C had increasing difficulty rewarming from 10°C to higher levels the longer they were heat exposed. If maximum difficulty to rewarm is synonymous with heat acclimation, bats reach full acclimation between 20–28 days of heat exposure (Figure). Bats exposed longer than 28 days (32–48) to heat found it somewhat easier to raise their internal temperature in the cold compared with bats exposed 20–28 days to heat. This partial reversal in the heat acclimation pattern might suggest a new thermal adjustment of the acclimated animal through changes in its central and/or peripheral

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.

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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.

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