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# PERIODICITY OF HETEROOTHERMY IN THE GARDEN DORMOUSE, *ELIOMYS QUERCINUS* (L.).

by

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## 1. INTRODUCTION

It is well known that the temporal organization of biological functions is largely based on endogenous physiological rhythms, which more or less match periodicities in the environment to which organisms are adapted. And to distinguish between proximate and ultimate causes of many biological phenomena, it will be necessary to understand their temporal organization. The study of natural mammalian hibernation has shown that hibernation is not a continuous process. Spontaneous interruptions occur throughout the hibernation season, and such arousals consume most of the energy available as stored resources for the whole overwintering period (KAYSER, 1952, 1961, 1964). Many hibernators neither feed nor drink upon waking up, and the physiological or ecological demands producing these interruptions are not readily apparent.

Long periods of continuous torpor in classical hibernators are at one end of a wide spectrum of mammalian heterothermic phenomena (BARTHOLOMEW, 1972), which range from daily torpor as found in many small rodents to hypothermic periods lasting more than 80 days

as observed in the Little brown bat, *Myotis lucifugus* (MENAHER, 1964). The number of bouts of torpor during a season of hibernation appears to be related to weight-specific metabolic requirements and the opportunities of a species to feed and to store body reserves (MORRISON, 1960; FISHER & MANERY, 1967; DAAN, 1973).

Variation in the length of torpor bouts is influenced by environmental temperature. Higher ambient and core temperatures lead to more frequent interruptions of hypothermia (KAYSER, 1952; PENGELLEY & FISHER, 1961; KRISTOFFERSON & SOIVIO, 1964a; TWENTE & TWENTE, 1965a, b). Even more variation is of seasonal origin, as evidenced by the observation that under constant environmental conditions in many species bouts of torpor are longer in midwinter than at the beginning and end of the hibernating season. In a few species this seasonal pattern is known to be part of an endogenous yearly rhythm (PENGELLEY & FISHER, 1963; PENGELLEY & KELLY, 1966; HELLER & POULSON, 1970). In the ground squirrel, *Citellus lateralis*, this circannual rhythm is innate since it also occurs in animals born and reared under constant laboratory conditions (PENGELLEY & ASMUNDSON, 1970). The nature of seasonal synchronization of hibernation rhythms by zeitgebers is not well understood (see reviews in PENGELLEY, 1967; KAYSER, 1970).

Part of the investigation reported here was set up to find out if also in dormice the hibernation cycle is based on an endogenous circannual rhythm, and how this cycle is affected by the normal seasonal course of temperature and photoperiod separately.

The circadian nature of daily torpidity has been described in a few small rodents (e.g., *Perognathus longimembris*, LINDBERG *et al.*, 1971). During hibernation in the bat *Myotis lucifugus* (MENAHER, 1959) and the dormouse *Glis glis* (POHL, 1961), metabolism and core temperature continued to oscillate in a circadian rhythm, freerunning under constant illumination and temperature for at least a few days. Arousals from hypothermia in the bats were found to be phase-locked to the rising part of the rhythm of body temperature (MENAHER, 1959). Yet, in a thorough review, KAYSER & HEUSNER (1967, p. 70) conclude that generally no systematic manifestation of a circadian rhythm is found in hibernation. Long lasting freerunning rhythms of arousal from hibernation under constant conditions could not be detected in most species (*Citellus lateralis*, TWENTE & TWENTE, 1965b; *Erinaceus europaeus*, KRISTOFFERSON & SOIVIO, 1964b; *Eliomys quercinus*, LACHIVER & BOULOUARD 1967; PAJUNEN, 1970). POHL's (1965) results in *Glis glis* are suggestive, but do not convincingly demonstrate such a rhythm.

Several questions can be asked concerning the functioning of circadian rhythms in torpidity. Apart from the uncertainty about arousals, it is

not known if also the onset of torpor is locked to a circadian rhythm. The phase relations of both onset and arousal to the activity rhythm have not been described in detail in long-term seasonal hibernators. It is known that ambient temperature has little effect on the period of the circadian rhythm of animals employing daily torpor, and thereby exposed to different core temperatures at least during part of the cycle (RAWSON, 1960; LINDBERG *et al.*, 1971). An intra-individual comparison of freerunning circadian periods in normothermy and heterothermy remains to be demonstrated.

In the experiments presently described activity and torpor of Garden dormice (*Eliomys quercinus*) were recorded both in conditions of entrainment by 24-hr zeitgebers and in conditions of freerun. It is appropriate to outline the sense in which several terms are used throughout the paper:

Normothermy = the condition of relatively constant, elevated body temperature.

Hypothermia, torpor, torpidity = the condition in which body temperature drops below the normothermic level and is largely determined by the ambient temperature.

A bout of torpidity (sometimes in short 'a torpidity') = one uninterupted period of hypothermia.

Heterothermy = the capacity to vary body temperature between normothermic and hypothermic level.

Arousal = the change from the hypothermic to the normothermic condition.

Arousal interval = the time between two successive arousals.

Hibernation = A more or less continuous succession of bouts of torpor longer than one day, typically occurring at one season in the animal's annual cycle.

$\tau$  = period of a circadian rhythm.

## 2. METHODS

Adult Garden dormice were purchased in September 1969 and 1970 from a breeding station (Station d'Acclimation et d'Eleveage, Bouillé St. Paul, Deux Sèvres, France). Each animal was kept in a wire-mesh cage (60 × 60 × 60 cm), to which a wooden nest box was attached. These nest boxes (Fig. 1) contained two floors. The upper level was accessible only through a corridor (5 × 5 cm in diameter) in the lower half. All animals exclusively used the upper part as their nest hole. This part of the box contained a platina thermistor fitted in a 50 × 4 mm copper tube. The thermistor was included in a Wheatstone bridge connected

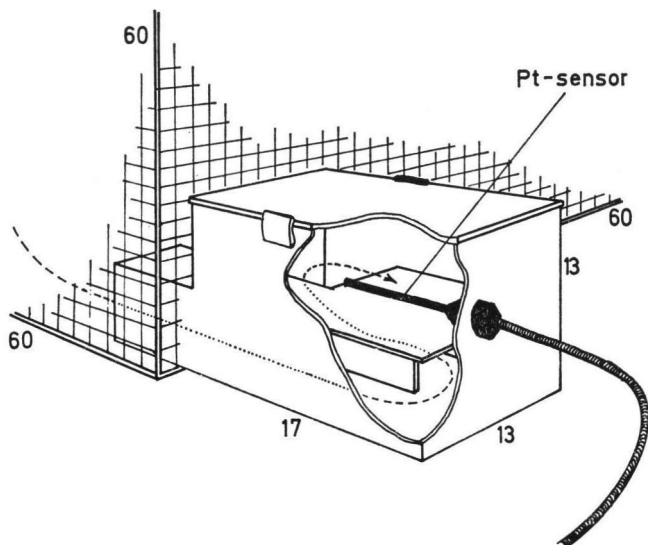


Fig. 1. Nestbox showing the position of the thermistor. Dimensions of cage and nestbox are given in centimeters.

to one channel of a 12-point potentiometric recorder (Philips PR 3120 U) and adjusted to cover a range of  $20^{\circ}\text{C}$  on the 25 cm scale of the recorder. Due to the spatial properties of the nest box, the animals were forced to build their nests on or close to the temperature sensor. Consequently, the temperature records largely reflected behavioural patterns of the dormice.

Fig. 2 shows a typical temperature record: a resting homeothermic

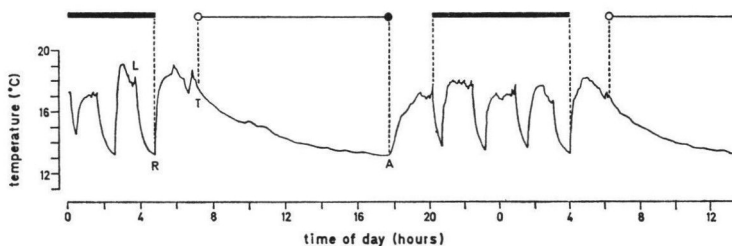


Fig. 2. Example of a nestbox-temperature record and its interpretation. L = Dormouse leaves the nestbox; R = dormouse returns; T = onset of torpor; A = arousal. Symbols used in subsequent figures for activity time and torpidity are shown above the record.

animal inside the nestbox produces a relatively high, more or less constant temperature. When the animal leaves the nesthole, the temper-

ature drops rapidly (L), whereas returning to the hole produces a sudden rise in temperature (R). The nocturnal activity period is characterized by a sequence of drops and rises in temperature. The initiation of torpor produces a much slower decrease in temperature (T) to the ambient level. The smooth form of the temperature curve at both beginning of torpidity and arousal (A), as well as the much longer intervals between these events clearly distinguish between torpor and absence from the nestbox. In pilot experiments, many checks all verified this interpretation of the temperature records. In all cases animals were found torpid when checked after a slow decrease in temperature, and were found active outside the nestbox when checked after a rapid decrease. In the analysis of data, activity periods were defined from the first sudden drop to the last sudden temperature rise of a daily series. Onset and end of a bout of torpidity were defined as the first visible slow temperature change leading to torpor and homeothermy respectively.

The periodicity of torpor and activity was studied in 24 animals, divided over four climatized rooms and one room exposed to outside temperature fluctuations (Table I). Throughout the experiments, the

TABLE I

Experimental conditions in five different rooms to which the five groups of garden dormice were exposed. Conditions in group IV and V were identical. Light cycles were artificial, without twilights between total darkness and illumination of *ca* 20 Lux.

Group	Animal No.	Period	Temperature	LD-cycle
I	1-4	1.XI.1969-21.IV.1971	12° C ± 0.5	12 : 12
II	5-8	1.XI.1969-21.IV.1971	12° C ± 0.5	seasonally varying (see Fig. 4)
III	9-12	1.XI.1969-21.IV.1971	outside fluc- tuations (see Fig. 4)	12 : 12
IV	16-18, 22-24	14.XI.1970-21.IV.1971	15° C ± 0.5	DD
V	13-14, 19-21	3.XII.1970-21.IV.1971	15° C ± 0.5	D

animals had access to food (pellets, apples, sunflower seeds, vitamins) and water *ad lib*. Food was replenished at irregular intervals and only during normothermic phases in the animals, to avoid any disturbance of torpor. In a few instances, nest boxes were treated with a flea repellent, to control excessive abundance of ectoparasites. Four out of 24 animals died in the course of the experiments. The others survived in apparently good health.

## 3. CIRCAANNUAL VARIATION

It was evident in most animals of groups I, II and III (cf. Table I) that bouts of torpor were longer in winter than in summer. This is exemplified by one animal of each group in Fig. 3. It is noteworthy that

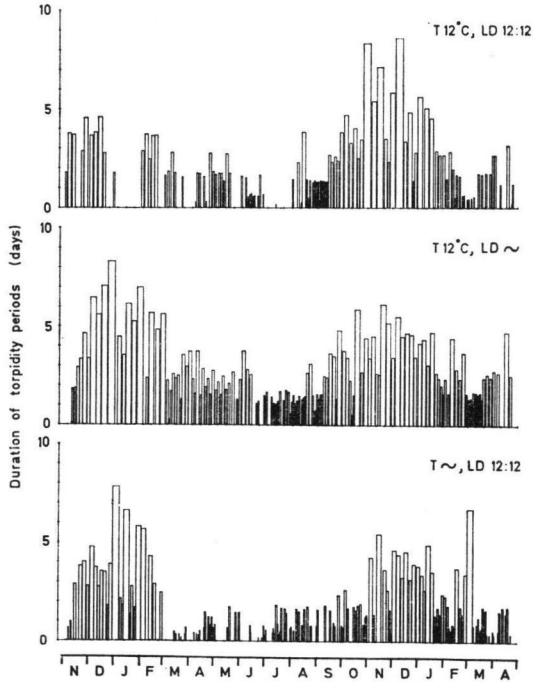


Fig. 3. Computer plots of torpidity durations as a function of time of year in a single animal selected from each of groups I, II and III (from top to bottom).

this seasonal variability occurred in three cases: (a) when photoperiod was constant and temperature fluctuated daily and seasonally (group III), (b) when temperature was constant and photoperiod was varied with season (group II), and (c) when both temperature and photoperiod were held constant (group I). Seasonal changes in mean duration of torpor are shown for all animals of groups I-III in Fig. 4. Most animals showed a decrease in length of torpor bouts during the summer of 1970, although the patterns are rather erratic. No distinct onset or end of the hibernation period can be detected. The most consistent annual fluctuation in duration of torpidity occurred in animals exposed to fluctuating temperature.

The results of group I (constant temperature and photoperiod)

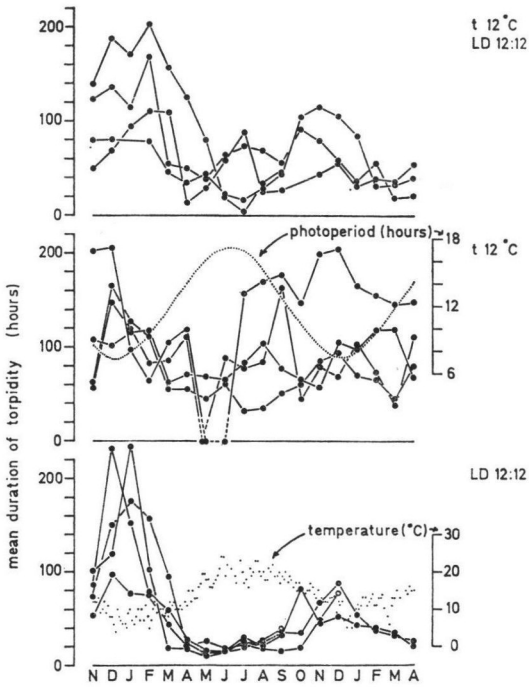


Fig. 4. Monthly mean duration of bouts of torpor in all animals of groups I, II and III (from top to bottom.) Changes in photoperiod are indicated for group II. Daily temperature readings at noon are shown for group III. Open circles indicate last values of animals that died during the experiment.

suggest that there is an endogenous circannual rhythm underlying heterothermy in the Garden dormouse. However, because torpor occurs throughout the year, this system is not as easily amenable to analysis as is the strictly seasonal torpidity of *Citellus lateralis* for which PENGELLEY & FISHER (1963) demonstrated a circannual rhythm by observing a clear onset to the winter season of heterothermy following a summer period of no torpor at all.

The monthly mean torpidity durations in the three groups in the first winter are compared with data of two previous studies in Fig. 5. The data of GABE *et al.* (1963) were collected by actography, and PAJUNEN (1970) used the "sawdust technique". Although both methods involve a larger range of inaccuracy in determining the times of onset and end of hypothermia than in the present study, they are suitable to assess the approximate duration of bouts of torpor.

All curves in Fig. 5 show the same pattern of seasonal fluctuation in duration of torpidity, although two of them were produced by animals



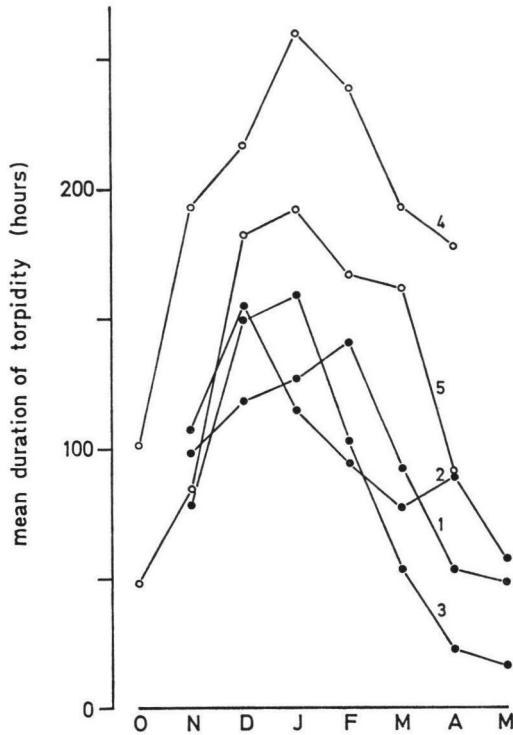


Fig. 5. Monthly mean durations of torpor during the season of hibernation. 1 = group I: LD 12 : 12, temperature 12° C. 2 = group II: LD varying, temperature 12° C. 3 = group III: LD 12 : 12, natural temperature fluctuations. 4 = results calculated from PAJUNEN (1970, Table 1): DD, 4,2° C; sawdust technique used, with checks 4 × daily. 5 = From GABE *et al.* (1963, Fig. I): natural photoperiod and temperature.

deprived from seasonal cues. There are, unfortunately, no field data to confirm the context in which this phenomenon occurs in nature. The monthly means extracted from PAJUNEN's data are approximately twice as high as those from the current experiments. This may be largely explained by the difference in temperature, which was 12° C in groups I and II, and 4,2° C in PAJUNEN's study. TWENTE & TWENTE (1965a) have presented an Arrheniusplot for the relation of duration of torpor to temperature in *Citellus lateralis*. Expectations of torpor duration derived from this relationship are 90 hrs at 12° C, and 180 hrs at 4° C. The ratio would account for the difference between PAJUNEN's and my results. It is not possible to tell whether the slightly longer torpor durations reported by GABE *et al.* (1963) can also be ascribed to temperature effects.

Summer torpidity is not well understood in dormice. It may occur as daily torpor or at least as bouts of torpor shorter than those in winter. GABE *et al.* (1963) report torpidity in *Eliomys quercinus* in July and August at environmental temperatures of 17-19° C. In the experiments described here, among twelve animals kept over 18 months, all except two (from group II in May and June 1970) showed instances of hypothermia at least once every month.

There is no simple explanation available for the deterioration of the magnitude of durations of torpor in the second winter.

#### 4. CIRCADIAN RHYTHMICITY

##### 4.1. Conditions of entrainment

The dormice of groups I, II and III were exposed to a daily light-dark cycle for a period of 18 months. In addition, group III lived under

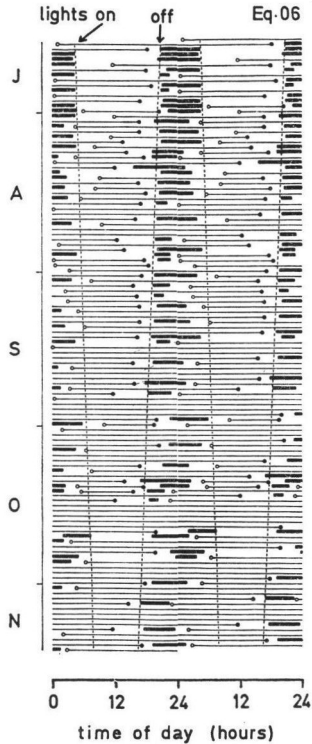


Fig. 6. Daily distribution of activity (black bars) and torpidity (thin lines) in a garden dormouse from group II, from July to November 1970. ○ = onset of torpidity, ● = arousal. Photoperiod is indicated by dashed lines. For clarity, the actogram is shown twice.

natural daily temperature fluctuations. Under these conditions, all animals showed a clear daily rhythmicity both in activity and heterothermy. Fig. 6 presents a four-month record for one of the dormice in group II (simulated natural photoperiod) as an example. Activity outside the nestbox is almost exclusively nocturnal. Torpidity occurs both during night and day, but onsets of torpidity typically occur between midnight and noon, whereas arousals from torpidity are centered a few hours before lights out. In addition Fig. 6 illustrates the gradual lengthening of torpidity periods from July to November.

The 24-hour distributions of 1098 torpidity onsets and 1021 arousals in all 12 animals of groups I-III are shown in Fig. 7. The two distri-

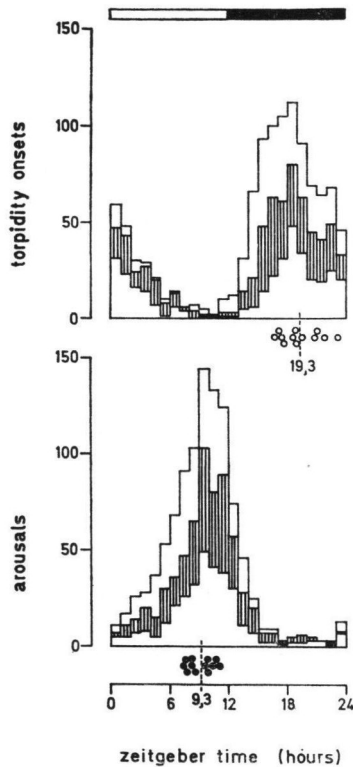


Fig. 7. Frequency distributions of torpidity onsets and arousals at hourly intervals. Zeitgeber time on the abscissa is defined by lights out at 12:00; Lights on is at 0:00 hrs in group I and III, and varies from 19:00 to 5:00 in group II. The frequency distributions for the three groups (I: lower area, II: middle, hatched area, III: upper area) are added. Mean vectors of onset (open circles) and arousal (solid circles) of the circular distributions in the twelve individual animals are shown. Dashed lines indicate mean vectors for all animals pooled.

butions are more or less complementary, with torpidity onsets occurring mainly during darkness and arousals mainly during light. The circular distributions of torpidity onset and arousal in all individual animals were highly significantly different from uniform (Rayleigh test;  $p < 0.001$ , see BATSCHLET, 1965). Mean onsets (Fig. 7) range from 16,7 to 23,3 hrs (total mean vector: 19,3; i.e. 7,3 hours after lights out). Individual mean arousal times range from 7,2 to 10,9 hrs zeitgeber time (total mean vector: 9,3; i.e. 2,7 hrs before lights out).

*Seasonal variation*

In an attempt to identify variables affecting timing of torpidity onset and arousal, seasonal variation and variation due to different duration

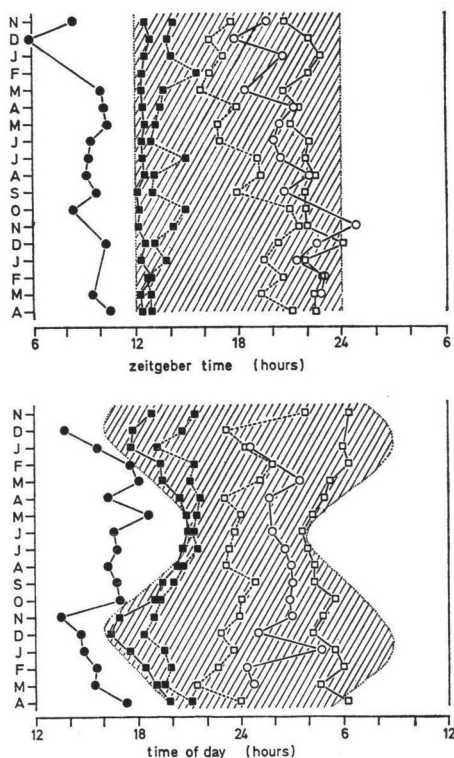


Fig. 8. Timing of torpidity and activity in groups I (top) and II (bottom), relative to the light-dark cycle. Time of darkness is indicated by shading. Symbols: ○ = mean vector of torpidity onsets, ● = mean vector of arousals. Mean vectors are only shown if the distribution departed significantly from random (Rayleigh-test,  $p < 0.01$ ). ■ = mean activity onsets, following torpidity (■---■) and following normothermic rest (■—■). □ = mean activity ends, preceding torpidity (□---□) and preceding normothermic rest (□—□).

of torpor were analysed. Fig. 8 shows monthly mean times for onset and end of both torpidity and activity for groups I and II, in constant temperature. Mean times of arousal and onsets of torpidity were calculated as the mean vectors of the circular (24 hr) distributions. Mean activity onsets and ends were calculated as the normal linear average as they have much narrower distributions. Distinction was made between activity onsets following torpor and onsets following normothermic rest. Likewise, activity ends followed by torpor were separated from the rest.

In group I (constant photoperiod of 12 hrs) there is no evidence of seasonal changes in the phase relationships of the various functions to the zeitgeber, although arousal and onset of torpidity gradually shifted backward in the course of the experiment. Arousal times were about 3 hrs before lights out, and torpidity onsets were about 5 hrs before lights on at the beginning of the experiment and shifted to ca 1 hr before lights out at the end of the experiment. Mean activity periods were about 10 hrs, starting ca 30 min after lights out throughout the year. There is, however a clear difference in onset and end of activity when they occur adjacent to a torpor cycle; such onsets of activity are delayed relative to the normal pattern by 1-2 hrs, and ends of activity are advanced by about 3-6 hrs.

In group II (seasonally varying photoperiod) there was a conspicuous seasonal fluctuation of the phase relationships with the changing LD-cycle. Activity onsets and activity ends followed the seasonal course of lights out and lights on respectively, though with lesser amplitude. Total activity time thereby varied less than did night duration. A similar pattern is known in Garden dormice in the natural daylight of summer (SAINT GIRONS, 1960) as well as in many other animals (ASCHOFF, 1969). Mean arousal times and subsequent activity onsets also follow "lights out" more or less, but appear less strictly coupled to it than normal activity onsets. The seasonal course of torpidity onsets and preceding activity ends seems to follow lights out rather than lights on.

#### *Torpidity duration and timing*

In Fig. 9, the relationship of the duration of a bout of torpor and its onset and arousal time is analysed. Bouts of torpor are pooled in groups, containing those lasting less than 24 hrs. ("1 cycle") 24-48 hrs (2 cycles), 48-72 hrs (3 cycles), etc. By plotting onset and arousal times for each group separately, a distinct pattern emerges. In Fig. 9 A, this is shown for one individual animal. Clearly, in this individual, the longer the torpidity the earlier in the day both its onset and its end occur. The circular means of the distributions shift significantly forward in time, with increasing torpidity duration up to 4 cycles long.

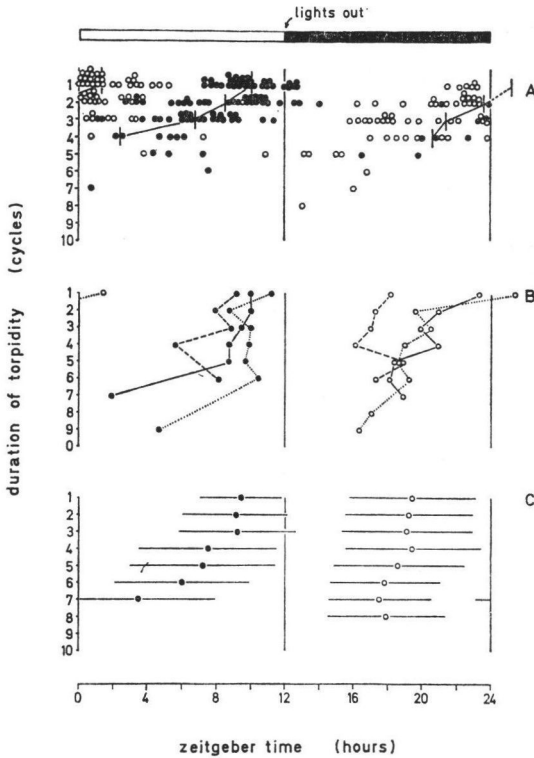


Fig. 9. Distribution of torpidity onset (○) and arousal time (●) as a function of duration of torpidity. A) Original observations for one animal (EqO2) from group I, in constant photoperiod and temperature. Vertical lines indicate mean vectors of distributions significantly different from uniform (Rayleigh-test,  $p < 0.01$ ). B) Mean vectors of significantly nonuniform distributions in groups I (—), II (.....) and III (- - -). C) Mean vectors of significantly nonuniform distributions in animals with constant photoperiod (groups I and III pooled). Horizontal lines indicate circular standard deviations (see BATSCHLET, 1965).

From 5 cycles onwards, the same trend is visible, but the distributions are no longer significantly different from uniform (Rayleigh-test,  $p > 0.01$ ), due to the small number of observations. The same tendency is present in the circular means of all observations pooled for each group (Fig. 9 B). The results for group II are included in this graph, but as the light-time changed in the course of the season, for these animals, the forward shift relative to lights out is here less convincingly related to torpidity duration. The results of group I and III (constant photoperiod), summarized to Fig. 9 C, confirm that both onset and end of long torpor bouts occur earlier in the day than onset and end of

short torpor bouts. Mean arousal from *daily* torpor (*i.e.*, 1 cycle) occurs later than arousal after longer bouts of torpor. Also, the variation of arousal times is smaller in the shortest torpidities. This tendency can also be inspected in Fig. 6, where arousal times seem to be less subject to variation in July-September, with relatively short bouts of torpor than in October-November, when torpor bouts last longer.

### *Torpidity duration*

The frequency distribution of lengths of torpor bouts for all 12 animals together shows a roughly exponential pattern (Fig. 10B), *i.e.*, short

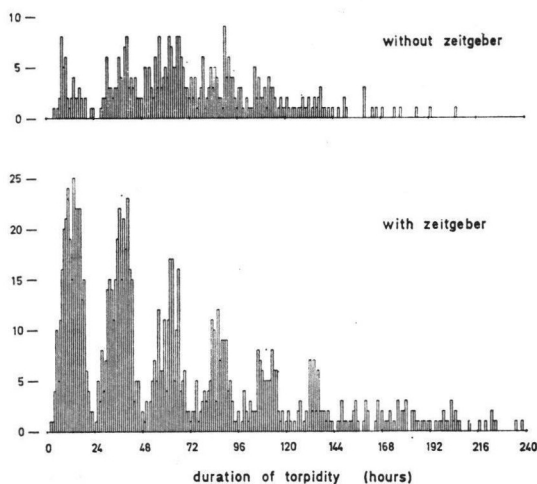


Fig. 10. Frequency distributions of durations of torpor. Groups IV-V (A, unentrained conditions, top) and groups I-III (B, entrained conditions, bottom) pooled.

periods occur more frequently than long periods. But the distribution is far from unimodal; at intervals of about 24 hrs the number of observations drops to nearly zero, whereas maxima are encountered at about 12, 36, 60 hrs etc. This means that, on the whole, a garden dormouse in torpor is most likely to wake up about 12 hrs after onset. If it does not wake up then, it is most likely to continue in torpor for an additional full cycle of its circadian oscillation, or some multiple thereof.

The shape of the frequency distribution of Fig. 10 B can be explained, and is in fact demanded by the 24-hour distribution in arousals and torpidity onsets (Fig. 7). Almost no arousals occur at the time of day when torpidity onsets are most frequent, and vice versa. The length of time between the maximum frequency of onsets and the maximum

frequency of arousals is slightly more than half a daily cycle. This produces the peaks in the frequency distribution (Fig. 10 B) at about 12 hrs and multiples of 24 hrs thereafter. Up to nine peaks at subsequent 24 hr intervals are visible, indicating that the underlying circadian rhythm persists through prolonged periods of hypothermia, at least in entrained conditions.

A more precise estimate of the periodicity involved in individual animals was obtained by autocorrelation of such frequency distributions as will be described in section 4.2. In all 12 animals periods between 21 and 27 hours were found (Table II, column C). Yet, the

TABLE II

Estimations of circadian periods ( $\tau$ ) during hypothermia and homeothermy. A) Values calculated from intervals between two consecutive activity onsets not immediately following hypothermia. B) Values calculated from intervals between consecutive arousals from hypothermia. For the calculation of  $\tau_B$  only such intervals were taken where neither the hypothermic phase nor the homeothermic phase lasted longer than three days. C) Values based on intervals between onset of torpidity and arousal.  $\bar{\tau}$  = mean  $\tau$ ;  $s_\tau$  = standard deviation;  $n$  = number of observations;  $p$  = significance level of differences between  $\tau_A$  and  $\tau_B$  (Mann-Whitney U-test);  $\tau^*$  = location of first peak in the autocorrelogram of the frequency distribution of interval lengths. Values of  $\tau^*$  based on peaks lower than 0.3 appear in parentheses. All values in hours.

<i>Freerunning</i>									<i>Entrained</i>		
<i>Animal</i>	<i>A) Normothermy</i>			<i>B) Normo/Hypothermia</i>					<i>C) Hypothermia</i>		
	$\tau_A$	$s_\tau$	$n$	$\bar{\tau}_B^*$	$\bar{\tau}_B$	$s_\tau$	$n$	$p$	$\tau_C^*$	<i>Animal</i>	$\tau_C^*$
Eq. 13♀	—	—	—	—	24.6	2.5	6	—	—	Eq. 01♀	23
Eq. 14♀	23.4	0.8	13	(24)	—	—	—	—	—	Eq. 02♀	24
Eq. 15♂	25.0	3.5	3	(25)	23.4	0.7	10	>0.1	(23)	Eq. 03♀	24
Eq. 16♀	24.1	1.2	12	25	25.0	0.8	24	<0.02	(27)	Eq. 04♂	(23)
Eq. 17♂	—	—	—	(22)	25.4	1.6	7	—	(26)	Eq. 05♀	(24)
Eq. 18♀	23.3	2.2	5	(24)	24.5	2.1	20	>0.1	(26)	Eq. 06♂	25
Eq. 19♂	24.2	0.6	55	24	23.8	1.0	33	<0.02	23	Eq. 07♀	25
Eq. 20♀	—	—	—	—	—	—	—	—	—	Eq. 08♂	22
Eq. 21♀	23.6	1.3	5	(21.5)	24.6	3.3	4	>0.1	(25)	Eq. 09♀	22
Eq. 22♀	23.8	0.7	12	(22)	23.7	1.3	18	>0.1	(23)	Eq. 10♀	24.5
Eq. 23♂	23.5	0.8	39	(25)	22.3	1.4	16	>0.1	(22)	Eq. 11♀	21
Eq. 24♂	23.2	1.0	13	24	24.3	0.9	29	<0.001	25	Eq. 12♂	27
All animals:				24					24		24

confidence limits for these estimations are not exactly known and probably wide. The deviations from 24 hours are therefore no proof that part of the animals were no longer entrained by the light-dark cycle when in hypothermia.



#### 4.2. Freerunning conditions

Both activity and torpor appear to continue in a circadian fashion in animals in constant conditions (Fig. 11). The rhythms freerun with

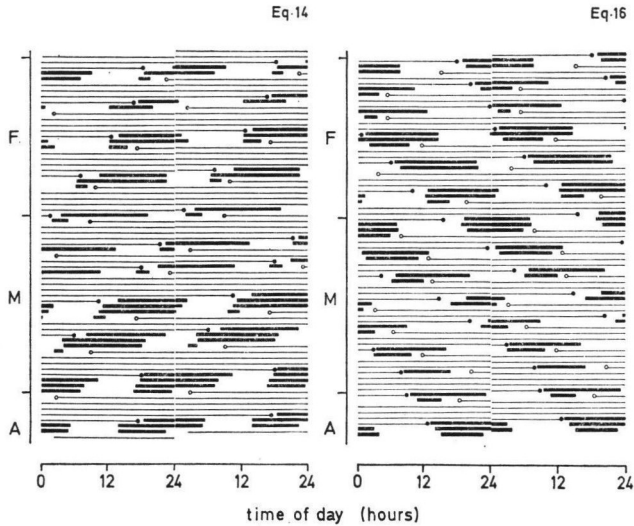


Fig. 11. Example of daily activity and torpidity in two garden dormice in constant darkness, February to April 1971. Symbols as in Figs. 2, 6. For clarity, the records are shown twice. A (left), Eq 14; B (right), Eq 16.

periods either shorter (Fig. 11 A) or longer (Fig. 11 B) than 24 hrs. In both examples several scans of the daily 24-hr cycle are completed, which is proof of the absence of entrainment and of the endogenous nature of the rhythm. The temporal organization of torpidity and activity relative to each other is generally the same as in entrained animals. Arousal from torpor occurs in the late “subjective day”, *i.e.*, in the time interval a few hours before the expected activity onset. The onset of torpidity usually occurs in the middle and late “subjective night”, *i.e.*, the time when the animal otherwise would have been active. By this arrangement, the activity pattern between two consecutive bouts of torpor typically consists of one or more complete periods of activity each followed by normothermic rest, and finally half a period of activity in the animal’s early subjective night. Often, the onset of the first activity after arousal is later than otherwise, as in entrained animals.

It is of interest to know whether torpidity itself affects the frequency of the circadian rhythm. The animal whose activity torpidity record is shown in Fig. 12 A is one of two animals out of 12 in the free-running groups that stayed normothermic for as long as several weeks at a time

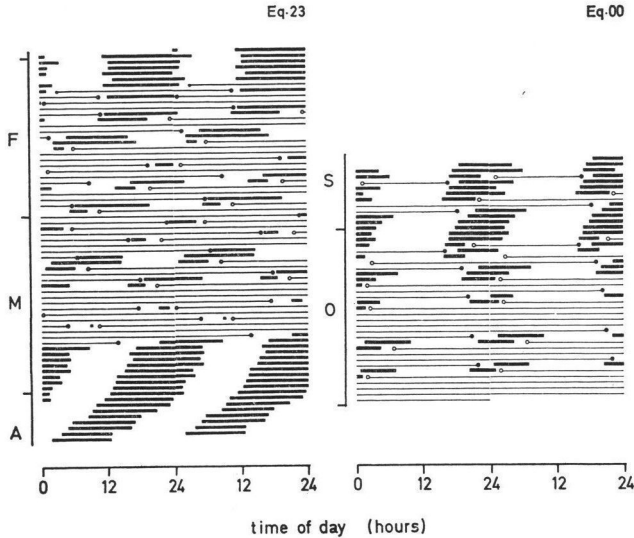


Fig. 12. Daily activity and torpidity in two garden dormice in constant total darkness. A, left: EqO3, February–April 1971, 15° C. B, right: An animal studied prior to the beginning of the main experiments shown in table I; September to October 1969, 12° C. Symbols as in Fig. 2.

during the winter. This record suggests that the underlying circadian rhythm had a much shorter period when torpor occurred (from February 6 to March 23) than in continuous normothermy. Before February 6  $\tau$  (as read from the onsets of activity) was about 24 hrs, until March 23 it was about 22.5 hrs, and after that date about 23 hrs. In another example (Fig. 12 B) hypothermia seems to have a delaying rather than an accelerating effect on the circadian rhythm. The period of the activity rhythm at the end of September was about 23.2 hrs. Each time torpidity occurred, the rhythm was phase-delayed by several hours. Over the whole record shown in this example, the average period thus became slightly longer than 24 hrs, even though in normothermic intervals the period was less than 24 hrs.

In order to investigate any possible systematic relationship between  $\tau$  and torpidity, actual values of  $\tau$  in both normothermy and heterothermy have to be measured. The estimation of  $\tau$  in normothermy can be done easily from the intervals between successive activity onsets (Table II, column A). Activity onsets tend to be subject to less variation than ends of activity under the experimental conditions. The first activity onsets immediately following arousal were not used in the average.

The measurement of  $\tau$  in torpidity confronts one with the problem

that arousals do not occur every circadian cycle and intervals between subsequent arousals are typically multiples of the circadian period. To avoid confusion, the word "interval" is used to denote the time lapse between onsets of torpidity and/or arousals, and the word "period" is restricted to circadian period. POHL (1967) has used an elaborate method to make estimations from these arousal-intervals in *Glis glis* by dividing them by integer numbers and grouping the quotients into classes characterized by minimum dispersion. However, this criterion is inconclusive, because some arbitrariness in the selection of permutations in the grouping can not be avoided. The procedure proposed by POHL involves extra weighting of the shorter intervals. However, any weighting applied at all should favour the long rather than the short intervals, since  $\tau$ -estimates from the former behave as means over a sample of cycles, and are thereby subject to less variation.

Unless one makes use of a continuously oscillating, circadian physiological variable, such as body temperature (MENAKER, 1959; STRUMWASSER *et al.*, 1967), to which arousal from hibernation is coupled, it is not possible to derive  $\tau$  from one individual bout of torpidity since one cannot be certain how many cycles are undergone during torpidity. Yet the stochastic properties of the interval lengths can be used to provide the additional information needed.

The frequency distribution of arousal-to-arousal intervals is typically multimodal in continuous total darkness, as exemplified by two animals (Fig. 13). The peaks in these distributions are roughly 24 hours apart. The same holds for the onset-to-arousal intervals (*i.e.*, durations of bouts of torpor). As in entrained animals, values of torpor duration lie near 12 hrs or in multiples of 24 hrs thereafter. This periodicity is also apparent in the accumulated histograms for all individuals (Fig. 10A), although less clearly, as would be expected if the animals ran free with different frequency. In the distributions of the onset-to-onset intervals, the circadian periodicity is likewise present, although less conspicuously than in the arousal-intervals, indicating that arousals are more tightly coupled to the circadian rhythm than onsets of torpidity. The difference is probably related to the slow cooling of the body and the thermistor when the dormice enter hypothermia, which allows a less precise definition than the rapid heating in arousal.

The multimodality of the interval lengths is in fact better evidence for the circadian rhythm than the gradual shifting away of activity and torpor in freerunning animals (Fig. 11). This would also occur when the arousal intervals were of nearly equal duration, *i.e.*, if they had a narrow Gaussian distribution around some mean interval length. The periodicity present in both the arousal intervals and torpidity durations has been analysed by the computation of autocorrelograms (*e.g.*,

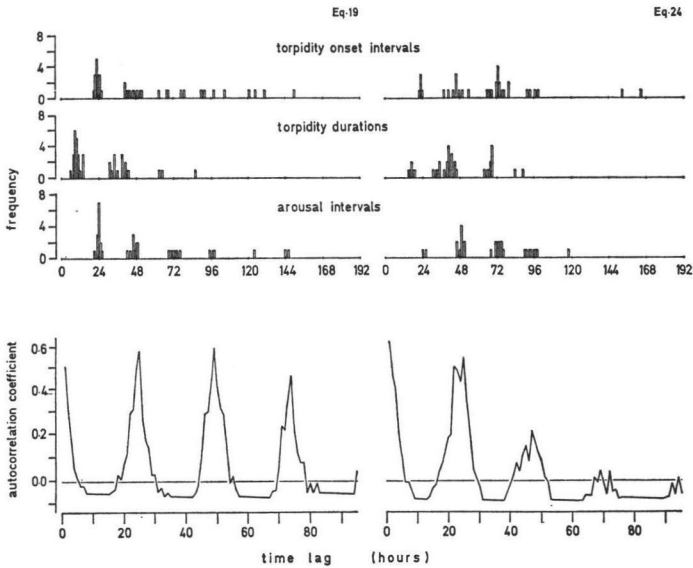


Fig. 13. Top: frequency distributions of intervals between two successive torpidity onsets, between torpidity onset and the next arousal, and between successive arousals, in two animals in constant darkness. Only intervals shorter than 8 days and certainly unaffected by incidental disturbances are shown. Bottom: Autocorrelograms of the frequency distributions of the arousal-intervals.

STRUMWASSER *et al.*, 1967) of the distributions (Fig. 13). The location of the first peak in a smoothed average of the autocorrelation function was taken as an overall measure of the circadian period ( $\tau^*$  in Table II, columns B and C). The circadian rhythmicity is demonstrated by peaks between 21 and 27 hrs in all animals.

The confidence limits for these overall estimates are unknown, and for a statistical comparison with  $\tau$ -values in normothermy it is necessary to use a different estimate. Therefore, individual  $\tau^2$ 's were derived from the arousal intervals by dividing each interval by the integer giving the quotient nearest to 24 hrs. The peaks in the frequency distributions of these intervals (Fig. 13) are thereby superimposed. Only short intervals were used, with neither the hypothermic nor the normothermic part exceeding 72 hrs, as with long intervals the range of possible variation around 24 hrs would become too much compressed by the division (Table II, column B).

Mean values of  $\tau$  in strict normothermy and in successive normo- and hypothermia were significantly different (Mann-Whitney-U-test;  $p < 0.02$ ) in three animals (Nos. 16, 19 and 24). It should be emphasized that these are the animals with the best original records and also the

most conspicuous multimodality in the frequency distributions of arousal intervals. Two of these were therefore selected as examples for Fig. 13. The precision with which  $\tau$  could be estimated in these animals is thereby relatively large ( $s\tau$  relatively small). Out of eight animals with enough values to allow a comparison, four had  $\bar{\tau}_B$  shorter than  $\bar{\tau}_A$  and four had  $\bar{\tau}_B$  longer than  $\bar{\tau}_A$ . In all these individuals,  $\bar{\tau}_B$  (normothermy + hypothermia) was intermediate between  $\bar{\tau}_A$  (normothermy) and  $\tau_C^*$  (hypothermia).

It must be concluded that the occurrence of hypothermia can effect slight, but in some cases significant, changes in the period of the free-running rhythm in constant darkness. However, the direction of the change varies among individuals. The statistical significance does not necessarily imply that torpor is causally involved in the difference in  $\tau$ . One might argue that torpidity and normothermy are not evenly spaced over the duration of the experiment, and that seasonal fluctuations of  $\tau$  could be involved. However, no evidence of a systematic change in the magnitude of  $\tau$  during the experiments could be found. Furthermore, the examples in Fig. 12 show that indeed immediate effects of torpor on the freerunning circadian period are found.

## 5. DISCUSSION

### *Seasonal variation*

The seasonal variation in duration of bouts of torpor, even under constant conditions, confirms PAJUNEN's (1970) findings. It disproves the statement of LACHIVER & BOULOUARD (1967) that no endogenous modulation is involved in the regulation of torpidity periods in the Garden dormouse. The seasonal pattern is in agreement with data reported for a number of other hibernators, both in captivity (*e.g.*, *Citellus* spp., STRUMWASSER *et al.*, 1964) and in nature (*Myotis* spp., DAAN, 1973).

In addition, the results show that the lengthening of bouts of torpor occurs again with the onset of the second winter. This indicates the existence of an endogenous circannual rhythm, although it is not proven that this is a self-sustained oscillation. One might argue that some uncontrolled environmental factor, fluctuating with a period of about a year, elicited the yearly fluctuation in duration of hypothermia. This is unlikely, especially since freerunning circannual rhythms of hibernation are now known in a number of species of *Citellus* (PENGELLEY & KELLY, 1966) and *Eutamias* (HELLER & POULSON, 1970). The finding of a circannual rhythm as the endogenous basis for the regulation of hibernation in the family *Gliridae* is of further interest, since

CADE (1964) has pointed out the likeliness of the polyphyletic origin of hibernation.

Apparently, there is some seasonal regulator at work which affects the duration of torpor in dormice throughout the year, producing a range of durations from about 5 hrs in summer up to 10 days in winter (at 12° C), and probably longer at lower environmental temperatures. It is beyond the scope of this paper to discuss whether summer torpor and prolonged hypothermia in hibernation are qualitatively identical phenomena. One might presume that summer torpor is less profound due to a larger body-to-ambient temperature difference, in a similar way as in the initial stages of hibernation in ground squirrels ("test drops" in *Citellus beecheyi*, STRUMWASSER, 1959) and some bats (*Myotis lucifugus*, HENSHAW 1970). Since evidence to the contrary is lacking for *Eliomys quercinus*, summer and winter heterothermy are treated as one phenomenon in the present analysis.

#### *Phase relations*

The circadian nature of the regulation of heterothermy in the Garden dormouse is clearly demonstrated. It has been reported previously (SAINT GIRONS, 1965; KAYSER, 1965) that arousals from hibernation in *Eliomys* tend to occur more often around dusk than at other times of the day, even though KAYSER (1965) did not detect circadian variation of other physiological parameters (body temperature, oxygen consumption, cardiac frequency) during hibernation in the species. In the present study, a similar typical time of arousal, centering around 2.7 hrs before lights-out was found. Also, onsets of torpor showed a clear diurnal rhythm, as they centered around 7.3 hrs after lights-out. Onsets and ends are nearly complementary in their daily phasing. Almost no bouts of torpor started in the last hour before lights out, when arousal frequencies were maximal, and vice versa. Onsets and ends of torpor were about 10 hrs —i.e., 150°) out of phase. However, entrance into torpor is a slower process than arousal, and the decrease in temperature in the nestbox is likely to lag 0-3 hrs behind the point where the animal's thermostat is turned off. Thus, the phase angle difference between torpidity end and onset may in fact be closer to 120° than to 180°. Most likely, the different time constants involved in the onset of and arousal from torpor contribute to the difference in their variability, and the fact that the variation of onset times is larger than of arousal times (Figs 6, 7) can not be interpreted to say that the circadian clock functions with greater precision in hypothermia than in normothermy.

In its phase relation to the activity rhythm, hypothermia is different from normothermic rest. Normothermic rest starts at the end of the dark period, or, in freerunning animals, at the end of the subjective

night. Torpor usually starts in the middle of the (subjective) night. The last activity period before the onset of torpor is thereby terminated much earlier in the night than other activity periods.

Long bouts of torpor tended to end earlier in the day than short bouts. Animals kept under constant photoperiod showed such a difference amounting to about six hours for bouts of torpor up to seven days (Fig. 9C). The simplest explanation would be that during hypothermia inside the dark nestbox, the animals did not synchronize their circadian rhythm to the light-dark cycle, and ran free with an average period of about 23 hrs. In the long run, the torpidity rhythm stayed synchronized, and synchronization must then have taken place during normothermic phases only. The question whether hypothermia itself or the stay in the dark boxes reduced the sensitivity to the light-dark cycle, remains unanswered. Torpidity onsets had the same trend of shifting forward with longer torpidities, though less extensively than arousals. Long bouts of torpor occurred in clusters throughout the experiments, and weaker synchronization during such periods would produce a more positive phase angle difference to the daily zeitgeber. That onsets are less affected by torpidity duration than arousals (Fig. 9C) indicates that synchronization occurs after arousal and before the next onset of torpor.

In animals in constant photoperiod no seasonal fluctuations in phasing of hypothermia relative to the light-dark cycle were apparent. In the seasonally varying photoperiod, both onset and end of torpor followed the seasonal course of lights-out rather than that of lights-on. Activity onset was also coupled to lights-out, and activity end to lights-on.

PITTENDRIGH (1973) has recently postulated the existence of a system of two coupled oscillators, one locking on to dawn and the other to dusk, as pacemaker of the circadian activity rhythm in higher vertebrates. This hypothesis has several attractive features lacking in previous models, among which are that it can account for the general bimodality of activity patterns, and the splitting under constant conditions. If a "night (N) - and "morning (M) - oscillator" are involved in the activity-torpor rhythm in *Elomys quercinus*, it is reasonable to suggest that the regulation of onset of and arousal from torpor is coupled to the N-oscillator. This would explain why animals entering torpor usually skip the second half of their activity phase, and why times of onset of and arousal from torpor correlate best with lights-out.

#### *Hypothermia and freerunning circadian period*

The absence of a seasonal trend in phasing with constant photoperiod is of further interest, since the phase angle difference between a biol-

ological oscillation and its entraining zeitgeber is an indicator of the spontaneous frequency of the biological oscillation (ASCHOFF & WEVER, 1962). Thus, from the observed phasing of torpor and activity in summer and winter, one would not expect a change in the freerunning period of the circadian rhythm.

Determinations of freerunning  $\tau$  in this study were only made in winter, and a comparison with summer is not possible. MENAKER (1961) found significant differences in the circadian periods of body temperature in *Myotis lucifugus* between summer and winter. In summer bats in torpor at 3° C he found a mean  $\tau$  of 22.4 hrs, and in winter bats  $\tau$  was 25.0 hrs. To my knowledge, MENAKER's is the only report of a seasonal change in freerunning circadian period in animals. Yet the evidence is inconclusive in that the summer bats did not arouse from torpor at 3° C, whereas winter bats showed one normothermic interval almost every day. MENAKER did not consider his data absolute proof of an annual cycle in circadian frequency unrelated to hibernation, although he excluded the possibility that his data are explained on the basis of a direct temperature effect. It will be shown that the differences can be related to the capacity of warming up to normothermy, and to temperature effects on the circadian clock.

The problem of the effects of heterothermy on the circadian period relates to its temperature compensation. Temperature compensation is one of the remarkable general properties of circadian clocks (SWENEY & HASTINGS, 1960), which is obviously necessary for accurate timing in organisms faced with large variations in body temperature. The clocks of endothermic animals would not need to be as precisely compensated as that of poikilotherms. However, RAWSON (1960) has shown that endotherm's clocks are equally well temperature compensated. Both the homeotherm *Peromyscus leucopus* under temporary artificial cooling and the heterotherms *Mesocricetus auratus*, *Eptesicus fuscus* and *Myotis lucifugus* had small values of  $Q_{10}$  in the effect of ambient temperature on the clock. In *P. leucopus* and *M. auratus*, RAWSON calculated the  $Q_{10}$ 's from phase delays in the onset of the activity rhythm caused by short (1-8 hrs) induced hypothermia. The  $Q_{10}$ 's in bats were measured by comparing freerunning periods at different ambient temperatures. The two bat species undergo daily torpor and their clock is thereby exposed to nearly ambient temperature once every day, although not through the whole circadian cycle. The  $Q_{10}$ -values given by RAWSON (1960, table 2) for bats, based on whole circadian periods, and ranging from 1.01 to 1.07, therefore underestimate the real temperature effect. The duration of daily normothermy is not specified in RAWSON's paper, but if one allows for 12 hrs of normothermy per cycle as a low estimate, one can say that the length-



ening of the period must have been brought about in the remaining, hypothermic part of the cycle. The  $Q_{10}$ -values thus calculated are distinctly higher than those given by RAWSON and attain averages of 1.13 (range 1.07-1.17) in *E. fuscus* and 1.12 in *M. lucifugus*. RAWSON's general conclusion that a temperature compensation of the circadian clock is present in endotherms is certainly not impaired by this recalculation. The point is made here, though, to draw attention to the conspicuous decelerating effect of low body temperature on the clock when it occurs at the normal time of daily torpor, *i.e.*, during the animal's subjective day.

If we try to explain MENAKER's (1961) results in summer and winter *Myotis lucifugus* in terms of differences in body temperature, there seems to be disagreement with RAWSON's (1960) data, which were partly based on the same species. Winter bats, although undergoing high body temperature once every circadian cycle, had large values of  $\tau$ , whereas summer bats, in continuous torpor, had short  $\tau$ . Yet, while in RAWSON's bats the effect of temperature during the subjective day was measured, MENAKER's summer and winter bats differed from each other in body temperature during their subjective night. It may well be that a differential response to body temperature during different parts of the circadian cycle is involved.

The hypothesis is advanced that high body temperature during the subjective day has an accelerating effect on the clock and high body temperature during the subjective night has a decelerating effect. It is of interest that the only phase response curve for temperature pulses measured in animals (in the pupal eclosion rhythm of *Drosophila pseudo-ZIMMERMANN et al.*, 1968, Fig. 3) shows that maximum advances are elicited by 12-hr pulses of high temperature from circadian time (ct) 22 to ct 10 (*i.e.*, nearly coinciding with the subjective day), and that maximum delays occur with pulses from ct 13 to ct 01 (*i.e.*, during the subjective night).

Phase response curves for temperature pulses in mammals and birds are unknown, and attempts to synchronize circadian rhythms of endotherms with temperature cycles have failed so far (DE COURSEY, 1960 in *Glaucomys volans*; BRUCE, 1960 in *Mesocricetus auratus*; HOFFMANN, 1969 in *Apodemus sylvaticus* and *A. flavicollis*). If the hypothesis explaining the data of RAWSON (1960) and MENAKER (1961) is correct, one would expect however that synchronization by temperature cycles is feasible in heterothermic mammals. Actually, R. G. LINDBERG (personal communication) has recently shown that heterothermic pocket mice (*Perognathus longimembris*) can be synchronized by temperature cycles with amplitudes as small as 3° C.

Returning to the results in *Eliomys quercinus*, we can discuss them in the

light of the hypothesis of differential effects of low body temperature. It was shown, both by phase shifts due to torpor in individual activity rhythms and by statistical comparison of  $\tau$  in normothermic activity rhythms with  $\tau$  derived from intervals between successive arousals, that the period of the freerunning rhythm can be significantly affected by hypothermia in either a positive or a negative direction. In all cases torpor occurred both during the subjective day (SD) and the subjective night (SN) of the animals. From the hypothesis no predictions of the effect of hypothermia lasting longer than 24 hours can be derived, since accelerating effects during SN and decelerating effects during SD may offset each other. Daily torpor, during SD only should always phase-delay the rhythm. This is in agreement with the relatively late arousal from daily torpor in entrained conditions. Daily torpor was rare in the freerunning *Elomys* studied in winter, and the results do not allow a firm conclusion.

The fact that  $\tau$  in the same species can both increase and decrease significantly under the influence of lowered body temperature is not easily explained on the basis of a single oscillator directing the pace-maker. However, this would be an easy feature to accommodate in the two-oscillator model proposed by PITTENDRIGH (1973). One could speculate that the "night oscillator" can only be decelerated by higher temperature, while the "morning-oscillator" can only be accelerated. The complementary temperature dependencies and the phase angle difference between the two oscillators would produce not only the phase response curve necessary for entrainment, but also the homeostatic protection of the period against temperature variations, as suggested already by PITTENDRIGH (1958). The ecological significance of such a mechanism in endotherms, which may reflect the general homeostasis of the clock rather than a special compensation for temperature, is obvious in hibernators, in that it will provide for keeping their activity rhythm in pace with the environment, even when the animals miss one or more activity periods.

## 6. SUMMARY

1. The periodicity of activity and hypothermia in *Elomys quercinus* was studied in 12 animals exposed to daily lightdark cycles for 18 months continuously, and in 12 animals exposed to continuous total darkness for 5 months in winter.
2. The occurrence of a systematic yearly fluctuation in the mean duration of bouts of torpor, both in conditions of seasonally varying photoperiod or varying ambient temperature and in constant photo-

period and temperature indicates that an endogenous circannual rhythm is involved in the regulation of duration of torpor.

3. Under the experimental conditions, at 12° C, bouts of torpor occur all year round and tend to be short in summer and longer in winter. No sharp onset or end of hibernation can be distinguished in the Garden dormouse.

4. Onset of torpor usually occurs in the middle of the (subjective) night, arousal from torpor in the late (subjective) day. With seasonally varying photoperiod both onset and arousal correlate better with lights out than with lights on.

5. Long bouts of torpor tend to start earlier and to be terminated earlier in the day than short bouts. Synchronization in the experimental conditions was probably restricted to the normothermic phases between bouts of torpor.

6. In the absence of a daily zeitgeber the rhythm of onset of and arousal from hypothermia freeruns with a period differing from 24 hrs. The persistence of the circadian rhythm during hypothermia is proved by peaks about 24-hrs apart in the frequency distribution of lengths of bouts of torpor.

7. Estimates of the circadian period in intervals between successive arousals differed from estimates derived from activity onsets in normothermy. Both significantly negative and significantly positive differences occurred, indicating that torpor may have both slight accelerating and decelerating effects on the circadian clock.

8. The hypothesis is advanced that low body temperature has a phase-delaying effect on the circadian rhythm of heterotherms during the subjective day, and a phase-advancing effect during the subjective night. The implications of such a mechanism for temperature compensation and entrainability by temperature cycles are discussed.

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