Comp. Biochem. Physiol., Vol. 62A, pp. 371 to 376
0300-9629/79/0201-0371$02.00/0

TORPOR INDUCED AT ANY SEASON BY SUPPRESSION OF FOOD PROTEINS IN A HIBERNATOR, THE GARDEN DORMOUSE (*ELIOMYS QUERCINUS* L.)

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(*Received* 22 *February* 1978)

Abstract—1. We have examined the influence of a protein-free diet on the behaviour of the garden dormouse.

2. The experiments performed during the active life of the animals show that protein deficiency (apple diet or synthetic protein-free diet) without fail induces torpor comparable to that provoked by starvation or to that occurring spontaneously during natural hibernation.

3. The delay before the first appearance of torpor and the length of the hypothermic phase in the garden dormouse is a function of the ambient temperature.

4. Torpor induced by protein deficiency occurs even though the energy requirements of the animal are amply satisfied.

5. Whatever the mechanism, it is probable that characteristic modifications of protein metabolism play a major role in the process of hibernation.

INTRODUCTION

It is generally agreed that both cold and the absence of food play decisive roles in the onset of hibernation (Mann, 1916; Wade, 1930; Lachiver & Kayser, 1958). In a study of the mechanism of hibernation in the garden dormouse, Ambid & Agid (1972) have shown that torpor can always be induced in this animal at any time of the year simply by depriving it of food, without any reduction of ambient temperature.

In order to distinguish the effects of total starvation (calorific and specific) from those due to the absence of a particular constituent of the food, we have investigated the relation between protein deficiency and the appearance of hypothermic phases. We have thus examined the influence of a protein-free diet on the behaviour of the garden dormouse.

MATERIAL AND METHODS

Adult dormice (*Eliomys quercinus* L.) of both sexes were kept in individual cages fitted with an actographic device for recording their activity. This gives an accurate indication of the length of the hypothermic phases. A central temperature control was also provided, the rectal temperature of each animal being recorded with the aid of a thermocouple. The experiments were performed at various periods of the year at ambient temperatures ranging from 16 to 30°C and with the normal photoperiod.

The recordings were made between May and October, the months during which these animals are active, for three consecutive years. Four different groups of animals were used, making 180 in all.

Group 1 contained control animals given normal rations, which consisted of unlimited synthetic rat food (UAR) together with a quarter of an apple a day. This diet has been used for many years and is completely satisfactory: with it, the animals remain in good health for as much as 8 years.

Group 2 contained animals given no food at all.

Group 3 consisted of animals fed exclusively on apples, in unlimited quantities. Dormice readily accept this diet since they are naturally fructivorous. Such a diet contains virtually no protein (0.3 g/100 g of fresh fruit).

Group 4 consisted of animals fed on synthetic UAR food in unlimited quantities completely free of protein, together with a quarter of an apple a day.

RESULTS

I. Caloric requirements

A full study of the energy consumption of these animals throughout the seasonal cycle has been made by Montoya *et al.* (1976); the energy requirements of the dormice, which exhibit considerable seasonal variations, were thus known when the experiments were in progress. The quantity of food eaten was carefully measured. The calorific value of each diet being known, we found that the energy requirements of groups, 1, 3 and 4 were very adequately fulfilled (Table 1).

The animals of groups 3 and 4 consumed more than those of group 1. This is explained by the fact that the dormice of groups 3 and 4 passed through hypothermal phases, interrupted by awakenings, which required very considerable energy expenditure. We have shown that a dormouse returning to normal temperature may consume as much as 8 l. O_2/kg hr; this figure drops to 1.5 l. O_2/kg hr once the temperature has become normal (Ambid, 1977).

II. The effects of protein deficiency on the activity of the dormouse

The experiments performed during the active life of the animals show that protein deficiency (apple diet or synthetic protein-free diet) provokes phases of hypothermia comparable to those induced by starvation and to those occurring spontaneously during natural hibernation, even though the calorific level of the diet is not reduced. In order to appreciate the influence of the various diets on the rhythm of activity

Table 1. Relation between the energy value of the food intake and the energy expended by the animals of the various experimental groups

Energy requirements (E.r.) of control dormice, homeothermic in June, in Kcal/24h. 100g body weight		Energy consumption (E.c.) during June in Kcal/24h. 100 g body weight					
		Group n°1 normal diet		Group n°3 apple diet		Group n°4 protein-free diet	
animal	(E.r.)	animal	(E.c.)	animal	(E.c.)	animal	(E.c.)
H 125	14,26	K 103	18,64	J 106	31,09	K 21	28
H 88	13,33	K 104	17,75	I 262	30,04	K 69	35,11
H 108	16,68	L 11	18,72	J 211	27,95	K 70	36,75
H 109	14,70			I 292	21,49	K 11	20
H 60	15,65			J 118	20,06		
I 184	14,20			J 105	37,64		
Mean	14,92		18,37		28,05		29,97
± S.E.M.	0,47		0,31		2,66		3,83

of the animals, we measured the delay between commencement of the diet and the onset of hypothermia and recorded the frequency and length of the periods of hypothermia that occurred during the subsequent weeks.

1. *Interval preceding first hypothermic phase.* We first examined the delay between institution of the various diets and the first appearance of hypothermia. This is the most spectacular demonstration of the rapidity with which protein deficiency provokes hypothermia. We have classed the dormice into three groups, those in which the first hypothermic phase occurred in less that 3 days, 3–6 days and more than 6 days after the beginning of the experiments (Table 2).

The majority of the animals entered a first hypothermic phase during the first 6 days of the experiment, during which 85% of the starved animals, 75% of the animals fed exclusively on apples and 81% of those fed on a protein-free diet fell into hypothermic torpor. At any given time of the year, all other things being equal, hypothermia does of course set in more easily the lower the ambient temperature. In our experiments this temperature varied from 16 to 30°C and the dormice invariably became torpid after a few days, sometimes after less than 48 hr, even during August.

2. *Length and frequency of occurrence of hypothermic phases for the various diets.* The times of appearance and the lengths of the various hypothermic phases observed during the experiments are plotted in Figs 2–4. The black rectangles represent the lengths of the torpid phases, which ranged from 8 to 72 hr, as indicated by the actograms. In view of the large number of records, we can present only a selection here. We therefore give the results of experiments performed during September (Figs 1–3) and of one August experiment (Fig. 4). In these cases, the data obtained are identical with those recorded at other active periods.

The starved animals and those fed on a protein-deficient diet (apples or synthetic protein-free food) passed through more hypothermic phases than the normally fed control animals, in which periods of torpor were less common. The torpid phases observed in control animals at the beginning of the summer can be attributed to the phenomenon of aestivation (Agid *et al.*, 1965), whereas those encountered in September and October are due to precocious prehibernation. In both cases, the animals lose their appetite and stop feeding prior to the onset of torpor. It is to be noticed too that total starvation has a more marked effect on the frequency and duration of torpid phases than the two protein-deficient diets. The latter had comparable effects on the rhythm of activity of the dormouse. Furthermore, it must be stressed that the ambient temperature during all these experiments never fell below 16°C and sometimes reached 30°C. The experiments performed during August showed that despite outside temperatures between 19 and 23°C, all the protein-deficient animals entered phases of torpor in 48 hr or less, whereas none of the controls exhibited hypothermia. These relatively high ambient temperature tend to discourage the onset of hypo-

Table 2. Delay before the first appearance of a hypothermic phase in dormice fed several different diets

Experimental conditions / Delay	0 to 3 days	3 to 6 days	more than 6 days
Starved (48 animals)	54 %	31 %	15 %
Apple diet (59 animals)	36 %	40 %	24 %
Protein free diet (27 animals)	37 %	44 %	19 %

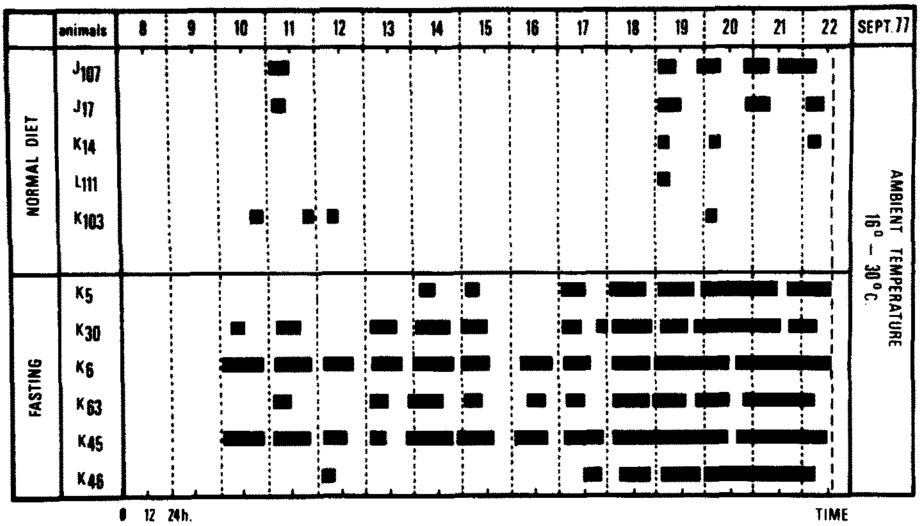

Fig. 1. The influence of fasting on the appearance of hypothermic phases in the dormouse. The lengths of the hypothermic periods (black rectangles) were recorded in two groups of dormice: animals fed normally and animals starved from the time when the recordings began. Starvation continued throughout the 15 days of the experiment.

thermia torpor in the dormouse. The higher the ambient temperature, the longer is the delay before the first appearance of hypothermic sleep and the shorter are the torpid phases.

3. *Magnitude of the phenomena.* We have attempted to study the observed phenomena quantitatively and statistically. We have therefore expressed the duration of the homeothermic phases of each animal (when the central temperature was approximately 37°C) as a percentage of the total length of the experiment, 2 weeks on the average. Figure 5 shows these percentages for the first and second weeks separately. During the first week, the starved animals spent $77 \pm 3\%$ of the time with normothermic body temperature whereas the corresponding figure for the control animals (group 1) was $95 \pm 1\%$. The homeothermic phases are thus 19% shorter in the starved dormice than in the controls during the first week. This result is extremely significant ($P < 0.001$). For the low-protein apple diet, the corresponding figure is $89 \pm 2\%$, 9.2% less than the controls ($98 \pm 1\%$) with $P < 0.001$. Finally, the protein-free diet gave $86 \pm 2\%$, 10.4% less than the controls ($96 \pm 1\%$), with $P < 0.001$.

When we examine the results of the second week, we notice an accentuation of the phenomenon of hypothermic torpor. The homeothermic phases of the starved dormice are now 47% less than the control value. The protein-deficient diets also produce much shorter homeothermic periods than the controls, 28% in the case of the apple diet and 27% for the protein-free diet. We see that the two protein-deficient diets have comparable effects on dormouse activity, the dif-

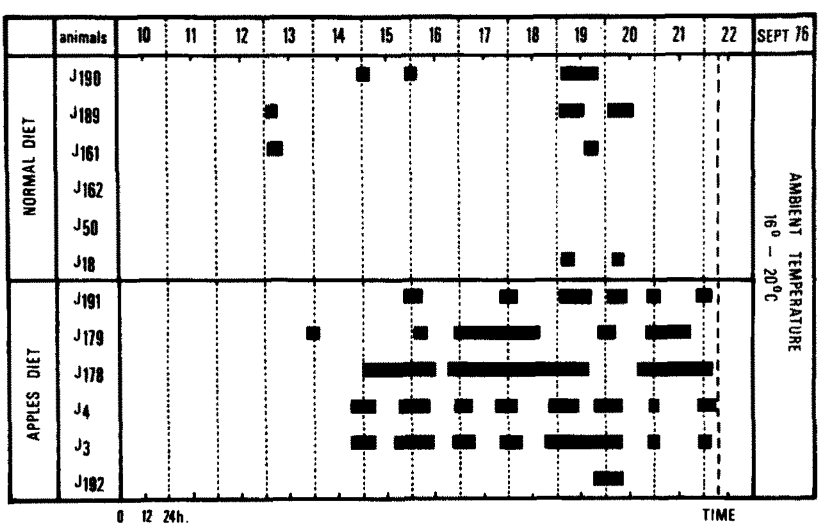

Fig. 2. The influence of a low-protein diet (apples only) on the appearance of hypothermic phases in the dormouse (same conventions as Fig. 1). The diet commenced at time zero and continued through the 14 days of the experiment. The animals fed only on apples passed through more periods of hypothermia which lasted from 8 to 72 hr.

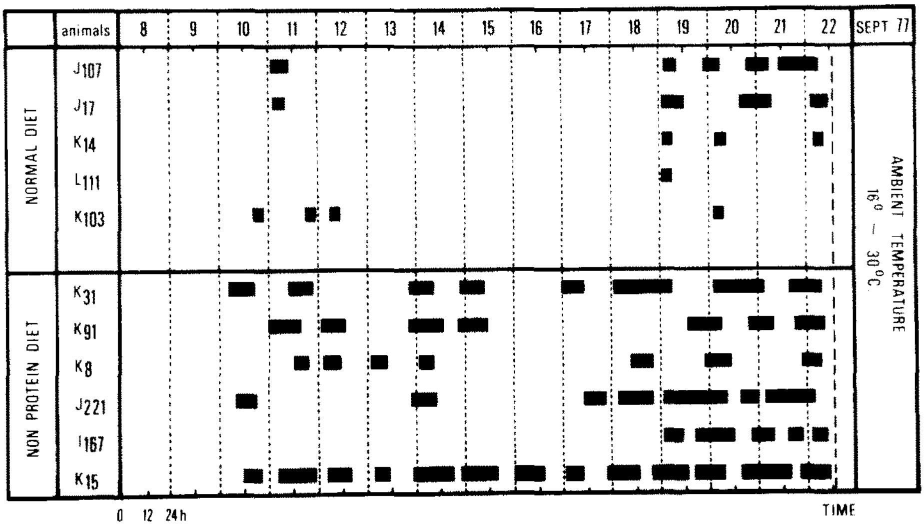

Fig. 3. The influence of a protein-free diet on the appearance of hypothermic phases in the dormouse (same conventions as Fig. 1). The diet commenced at time zero and continued for the 15 days during which readings were taken. The animals fed on a protein-free diet passed through more hypothermic phases, which lasted for 6–72 hr as opposed to 6–15 hr for the controls.

ferences observed are not significant. These diets cause a drop in the total duration of homeothermic state of 8–10% during the first week and 25–30% during the second week. These findings are statistically different from those obtained with total starvation, where the drops are around twice as large (19 and 47%).

The first week of the diet thus initiates a progressive lowering of body temperature towards ambient levels which is more rapid during the second week as the duration of torpor becomes severe. The fact that the drop in the duration of homeothermia was not greater should not be regarded as surprising since the duration of the torpid phases and the frequency of arousal are governed by the ambient temperature which remained high. In these conditions, the diets cannot induce extended torpor but instead, we observe a succession of short torpid phases, terminated by frequent arousals.

DISCUSSION

Protein deficiency leads to reversible hypothermic torpor, comparable with that provoked by starvation or occurring naturally during hibernation, whether the diet consists wholly of apples or of synthetic protein-free food. Torpor induced by protein deficiency occurs even though the energy requirements of the animal are amply satisfied. From Table 1, energy intake and energy expenditure are balanced, in the case of the control animals: intake (18.38 kcal/24 hr 100 g body weight) scarcely exceeds expenditure (14.92 kcal/24 hr 100 g body weight) for animals in the same conditions. For the protein-deficient animals, however, intake is distinctly higher (28.05 kcal/24 hr 100 g body weight for the diet and 29.97 kcal/24 hr 100 g body weight for the synthetic protein-free diet). This can be explained by the fact that the protein-deficient animals pass through a succession of hypothermic phases terminated by arousals. During hypothermia, the energy expenditure is related to the body temperature, which is in turn governed by the ambient temperature. In our experiments, the latter was quite high so that even during hypothermia the energy expenditure was by no means negligible. Furthermore, considerable energy is consumed during the periodic awakenings. Overall, therefore, the protein-deficient animals use much more energy than the homeothermic control animals. Their energy intake is also much higher, however, and the hypothermic phases cannot be attributed to a shortage of energy.

This hypothermic torpor occurs during the active life of the animals at a time when the controls show normal activity, despite ambient temperatures always above 16°C and sometimes as high as 30°C, which certainly does not favour hypothermia. These phases of lethargy, during which the central temperature of the animal is equal to or very slightly above (0.5°C) the ambient temperature, occur after a certain delay and with a variable frequency both of which vary

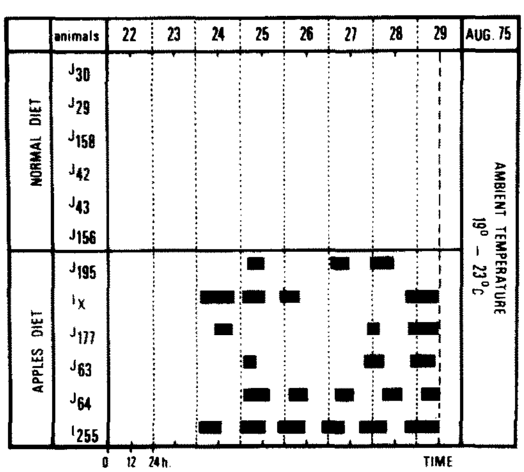

Fig. 4. The influence of a low-protein diet (apples), on the appearance of hypothermic phases in the dormouse during August (see the caption to Fig. 2)

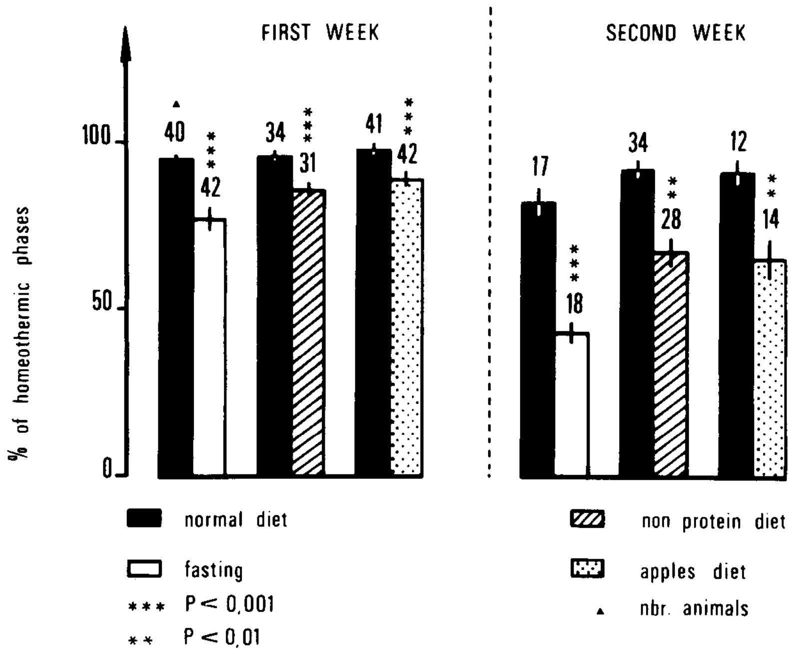

Fig. 5. The influence of starvation or protein deficiency (caused by an apple diet or a protein-free diet) on the duration of the homeothermic phases in the dormouse during the first and the second weeks of the experiment.

with the ambient temperature. The two protein-deficient diets have comparable effects, less pronounced than total starvation as far as both the delay before the occurrence of the first hypothermic phase and the total duration of these phases are concerned.

Major metabolic and endocrinological changes occur with hibernation (Kayser, 1961). In the hibernating dormouse, the metabolism is considerably altered (Ambid *et al.*, 1971); these observations are in agreement with the changes in endocrine activity, the cessation of adrenal activity in particular, observed by Gabe *et al.* (1963) in the same species. Comparable results have been found after fasting at any season and any ambient temperature; after 4 days of starvation, a considerable hepatic steatosis develops (Ambid & Agid, 1972), adrenal activity ceases (Ambid & Montoya, 1976) and there is a reduction of O_2 consumption in the hours preceding the appearance of torpid phases (Montoya *et al.*, 1976).

Our preliminary results indicate that the hepatic disorders are due to some disturbance of the protein metabolism associated with defective lipoprotein synthesis (Ambid & Montoya, 1976). Furthermore, the lipid and hormone disturbances that occur during natural hibernation, which can be provoked experimentally by starvation, also seem to be induced by protein deficiency, according to our recent findings.

In the induction of hypothermia by starvation, the decisive element seems to be the lack of protein since the phenomenon can be reproduced by simply withdrawing proteins, while providing for the energy requirements of the animal. Calory shortage does of course accelerate and accentuate the induction of hypothermic torpor. The latter could be due to the absence of alimentary proteins and the inability of the animals to call on their protein reserves at least in adequate quantities. The induction of torpor could be a consequence of some disturbance in protein metabolism.

This hypothesis, which need experimental confirmation, is supported by the fact that the hypothermic torpor is always accompanied by an excess of hepatic lipids, which is connected with a disturbance of the β lipoprotein metabolism. The mechanisms culminating in hypothermia are not known. However, the preliminary results showing that the adrenals became inactive and there is a reduction of oxygen consumption in the hours preceding the phases of torpor, suggest a chain of cause and effect is in question. This would imply that the protein deficiency leads to inhibition of the sympathetic tonus, which in turn facilitates the onset of hypothermia. This suggestion is in complete agreement with the work of Draskoczy & Lyman (1967), who detected a halt in brain catecholamine turnover in the ground squirrel in the hours prior to hypothermia, even before there was any drop in central temperature. This suggests that a stoppage in the functioning of the adrenergic neurones could be at the origin of the onset of hibernation. Other results concerning hibernation itself favour such an interpretation: the endogenous level of hypothalamus catecholamines drops in the ground squirrel *Citellus undulatus* at the onset of hibernation (Feist & Galster, 1974) while in the hibernating hedgehog, the mechanisms of capture and or storage of noradrenaline in the central nervous system are inactivated (Faure & Calas, 1977).

Our work, being limited to the qualitative aspect of the diet, raises the problem of the spontaneous onset of natural hibernation. During the few days preceding hibernation, the animals have little appetite and stop eating. The immediate consequence is a protein deficiency which soon leads to a disturbance of the protein metabolism since the animal appears incapable of calling on its protein reserves, in sufficient quantities at least.

This disturbance in the protein metabolism could reduce the activity of the central adrenergic neurones which are known to play a fundamental role in maintaining homeothermia (Bligh, 1966). Such a hypothesis explains the observed drop in sympathetic tonus in terms of the inactivity of the adrenal and reduced respiratory exchanges in the hours preceding hypothermia.

Our experiments were restricted to the garden dormouse but the same mechanism could well exist in other hibernators, this merits further investigation. In conclusion, we believe that the seasonal variations in appetite are at the origin of the decisive changes that govern the loss or maintainance of homeothermia in the dormouse. But what are the causes of the spontaneous variations in appetite in many hibernating species? This question remains open.

Acknowledgements—Our thanks are due to M. J. Guinaudy for her assistance in preparation of the figures.

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