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Mammalian Hibernation [and Discussion]

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Mammalian hibernation

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In mammalian hibernation, the body temperature approaches that of the surroundings, allowing large savings in energy costs of basal metabolism and eliminating the need for heat production to compensate for heat loss. During entry into hibernation, heat production ceases while the body temperature set-point gradually decreases during slow-wave sleep. In the hibernating phase, the animal copes with problems concerning the maintenance of ion gradients, possible membrane phase transitions and the risk of ventricular fibrillation. In the arousal phase, the main part of the heat and practically all the necessary substrate comes from brown adipose tissue. The hibernation season is preceded by a preparatory phase. It may be concluded that hibernation is a practical, and perhaps even enviable, solution to a mammalian problem.

Introduction: what is hibernation?

In our normal encounters with mammals of all kinds (including our fellow humans) we have been accustomed to associate life with the feeling of the warmth of the body. The existence of a state of mammalian life in which a mammal can be found cold as if in a cold store, immobile and apparently even without breathing is therefore fascinating. That such quasi-dead mammals can regain bodily warmth and full activity and that they do this without the help of any 'external' heater is indeed one of the wonders of nature.

For this, as for other phenomena of nature, the fascination transpired into scientific investigation. But alas, hibernation is an evasive state: it occurs only amongst certain species of mammals, most of which are not among the most common laboratory mammals; in nature you rarely meet the hibernating hibernator because it is normally buried in its hibernaculum beneath feet of snow and earth; hibernation is a seasonal event that only occurs when the animal is in a certain 'mood', and even if it should be in that mood there is no way of experimentally inducing an animal to actually hibernate; if you are lucky to have a hibernator and it is hibernating, its response to any investigation is (fully in accordance with Heisenberg's uncertainty principle) that it will cease its hibernating activity, arouse and become similar to the rest of us. For these reasons, in hibernation research, we are still largely left with the fascination of the unknown.

Some of the features of the hibernation process can be followed on figure 1, which shows the body temperature of a hibernator during a hibernation bout. It is seen how the recording starts with an indication of a normal body temperature (a peritoneal temperature of nearly 37 °C in the hibernator in question, how a phase follows (called the entry phase) in which the temperature slowly decreases and approaches the temperature of the surroundings (6 °C), how during the true hibernation phase the body temperature is low and constant at about 6–7 °C and how, after several days in this condition, the hibernator spontaneously starts to rewarm

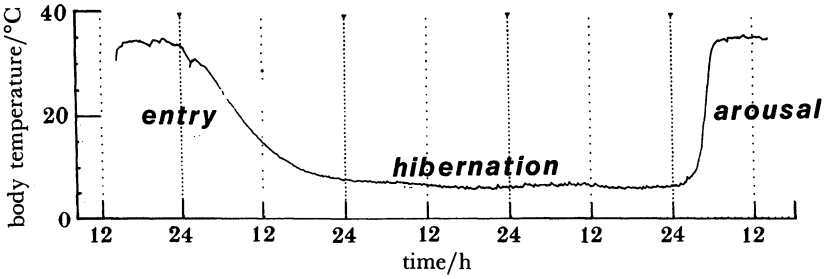

FIGURE 1. Recordings of body temperature during a hibernation bout. The phases of 'entry', 'deep hibernation' and 'arousal' can be clearly distinguished. Note that the total bout only lasted for about 3 days. (Based on recordings of Pévet *et al.* (1989) done on the European hamster.)

and rapidly regains normal euthermic temperature (while still in the cold), in what is known as the arousal process.

We shall briefly outline some of the knowledge that has accumulated on the hibernation process and some of the (many) problems remaining unsolved in hibernation research. For thirty years now, a series of meetings devoted to the hibernation process have been seminal to all research in the field, and the series of books that have emanated from these meetings remains an eminent source of both general knowledge and detailed investigations in the field (Lyman & Dawe, 1960; Suomalainen, 1964; Fisher *et al.* 1967; South *et al.* 1972; Jansky & Musacchia 1976; Wang & Hudson 1978; Musacchia & Jansky 1981; Heller *et al.* 1986; Malan & Canguilhem 1989). Also a recent book (Lyman *et al.* 1982) and some shorter review articles (Wang 1987, 1988, 1989) have been published. For more detailed information we refer the reader to these sources.

Why hibernate?

As the word implies, hibernation is a winter activity. In the septentrional parts of the world, the winter remains the season where there is a scarcity of food. Whereas the birds may leave for areas of the world where life is easier and food is abundant, terrestrial life has to survive these winter months on location.

For a mammal with a body mass of, for example, 1 kg, the basal metabolic rate is about 3.34 W. Thus even to sustain life at this modest level the animal has to accumulate food, before winter comes, from which to obtain sufficient energy to furnish its metabolic activities when no food is available. If we base calculations on a short winter of 100 days (and often they are much longer), the animal would have to have accumulated 29 MJ of energy. If this were to be carried on the mammal itself, it would add in lipid mass alone a further 961 g to the 1000 g.

The situation is even worse than this. The calculations above refer to basal (or minimal) metabolic rate; this rate demands that the mammal is under thermoneutral conditions. For most smaller mammals of the kind we discuss here, thermoneutral conditions mean environmental temperatures above 20 °C; for the smallest mammals even above 30 °C. Thus, on a winter's day, the mammal will be exposed to a cold stress and it would have to increase its metabolism several-fold to counteract heat loss to the surroundings (figure 2). In reality, we should therefore add several kilograms of fat to that already calculated above as the minimal requirement to furnish the energy for this extra heat production. The hibernator, instead of struggling to keep its temperature at 37 °C, allows it to drop to that of the surroundings, gaining doubly by this. Firstly, it does not have to use extra energy to counteract heat loss.

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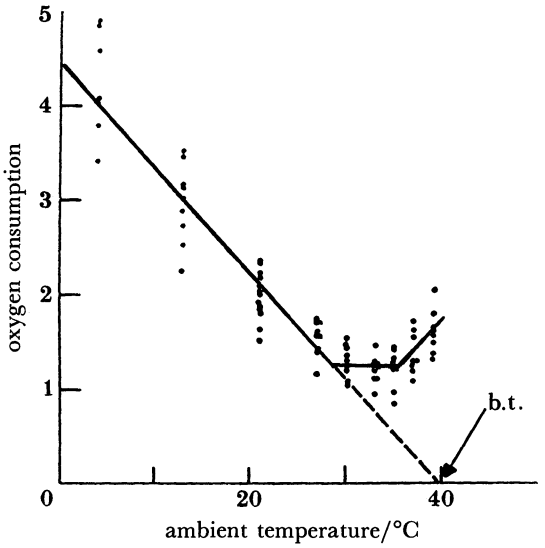


FIGURE 2. Effect of environmental temperature on metabolic rate in homeothermic mammals. Oxygen consumption was monitored in the Eastern chipmunk (*Tamias striatus*) as a function of environmental temperature; b.t. body temperature. (Modified from Neumann (1967).)

Secondly, it reduces its metabolism because of the Q_{10} effect. The reduction in the rate of a chemical process with temperature is two- to threefold for a 10 °C interval (a Q_{10} of 3) (figure 3). Thus, when the hibernator reduces its body temperature from 37 °C to about 7 °C, it can count on a 10–30-fold reduction in metabolism, resulting in a 10–30-fold reduction in the energetic price of a winter (provided that it stays dormant for the whole winter). In reality, the reduction in metabolic rate is often even more profound than would be expected from a simple Q_{10} effect. This is partly because, as an effect of the lowered temperature, the pH of neutrality is increased, and there is therefore a tendency to an 'acid inhibition' of metabolism (Malan 1982).

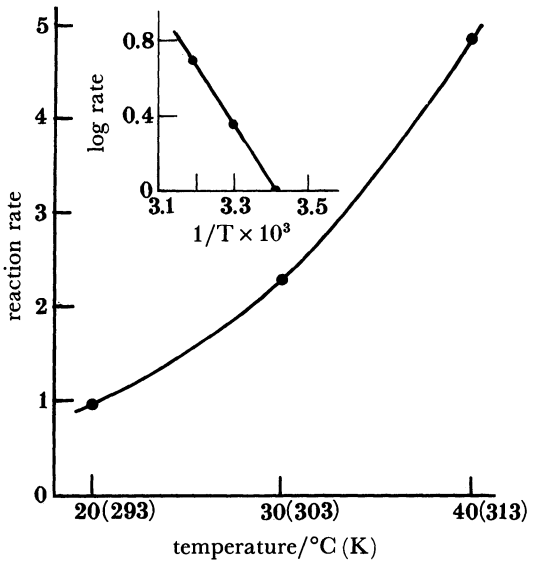


FIGURE 3. The Q_{10} effect. Theoretical diagram showing relative metabolic rates at different temperature, based on a Q_{10} of 2.5. The insert shows an Arrhenius plot of the same points. The slope of the plot is $-E_a/R$, where E_a is the activation energy and R the gas constant. (Adapted from Cannon & Johansson (1980).)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Why don't we all hibernate?

Although it would seem beneficial for any mammal to hibernate, there is apparently a price to pay for this. One problem is, of course that to reinstate life at normal body temperatures, you have to rewarm from the cold, an effort that demands substantial amounts of energy. Also other, perhaps costly, adaptations of metabolism may be necessary. Here, as elsewhere, it would seem that Nature has made a cost-benefit analysis of the process, with the result that it did not endow all mammals with the ability to hibernate.

One important factor is body size. It has long been known that there is not a direct proportionality between body mass and basal metabolism. This is true for all forms of life: bacteria, plants and animals alike. Thus, in a comparison between species, a tenfold increase in body mass does not lead to the expected tenfold increase in metabolic rate, but only to an approximately fivefold increase (for a review, see, for example, Schmidt-Nielsen 1984). In mammals, the relation can be expressed by:

$$\textit{metabolic rate}\ (W)\ \textit{of an animal} = 3.34 \times (\textit{body mass}\ (kg))^{0.75}.$$

We can therefore calculate (table 1) the cost of a winter for animals of different sizes. It is evident from the table that with increasing size, the amount of energy to be stored becomes smaller and smaller relative to the body mass, and that at least in animals larger than man the cost of a winter is no longer insurmountable. Thus, for a mammal as large as a bear, the value of going into true hibernation starts to become dubious.

TABLE 1. EFFECT OF BODY SIZE ON HIBERNATION FEASIBILITY[a]

(Because of the decline in specific metabolic rate with increasing body mass, the total amount of energy that has to be saved before a winter is relatively smaller for a larger animal than for a smaller one. In this table, theoretical values have been calculated. The calculations are based on a winter period (without food) of 100 days and a basal metabolic rate obtained from the formula $3.34 \times (\text{body mass})^{0.75}$ (see text). From this, the total 'winter price' is obtained: this is the number of joules that need to be accumulated before the winter in order to remain euthermic without food for these 100 days. Principally this energy could be available either as a store of food or in the form of fat in the animal. The amount of 'fat needed' is thus obtained by dividing the 'winter price' with a caloric equivalent of 30 kJ g^{-1} fat and this amount is expressed relative to the body mass in the last column. It is seen that before winter a small animal may have to carry three times as much fat as its own lean body mass, whereas a large animal only needs a small fraction of its lean body mass.)

body mass / kg	animal	Winter price / MJ	fat needed / kg	body mass (% extra)
0.01	mouse	0.95	0.032	320
0.1	rat	—	—	—
1	—	29	0.961	92
10	marmot	—	—	—
100	man	—	—	—
1000	bear	5134	171	17

So, do bears hibernate? Of course, in colloquial terms, they do. But, in reality, they have taken the consequences of the relations discussed above and they never progress into what in hibernation research is understood as 'true' hibernation: during winter their body temperature does not decrease much more than it does during normal sleep, and the bear remains active and alert the whole winter in its den. If it is female, it both gives birth to its cubs and nourishes them through the winter in this 'hibernating' state. However, metabolic adjustments to a life without food occur. Recently these adjustments have been compared to those occurring in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

humans undergoing prolonged reductions in food intake, such as in anorexia-nervosa patients (Nelson 1989). Also other large mammals living under conditions with a scarcity of food (such as the Svalbard reindeer) have 'chosen' not to hibernate but only to make metabolic adjustments to a situation with a severe food scarcity (Blix 1989).

Thus no mammals larger than a marmot (about 5 kg) are known to hibernate, but for smaller mammals, the incentive to hibernate becomes progressively greater. A further reason for this can be deduced from the calculations in table 2. As stated above, an obvious price that has to be paid for a hibernation bout is that of rewarming. Thus if the time spent dormant in a hibernation bout is short, it may cost more energy to rewarm from the cold than has been gained by the reduced metabolism in the dormant state. The values calculated in table 2 represent the break-even hours, i.e. the rewarming energy calculated as the number of hours of metabolism at euthermia that could instead be obtained for the same energy cost. For the heavier animals, hibernation bouts have to exceed several days before they become energetically meaningful, but for a very small mammal even a few hours of 'hibernation' pays. This fits well with the realization that amongst the smallest mammals, such as normal mice (which are not hibernators in the usual sense) it is quite common to observe daily torpor, especially in a situation where food is scarce. This daily torpor is reminiscent of hibernation in that during normal sleep, mice will allow their body temperature to approach that of the environment (Webb *et al.* 1982).

TABLE 2. REWARMING EQUIVALENT IN RELATION TO BODY SIZE

(One price that has to be paid for hibernation is the energy cost of rewarming. This cost is high compared to that of staying euthermic, but because of the effect of body size on specific metabolic rate, the cost is relatively higher in larger animals. This table presents a theoretical calculation of the relative cost of rewarming. The calculation is based on the energy needed to rewarm the entire animal 30 °C (from 7 °C to 37 °C), assuming a specific heat of 4.2 joules per gram per degree (i.e. that of water). By division with the basal metabolic rate of the animal of that size, the rewarming equivalent (in hours) for each animal size is calculated. This value represents the number of hours the animal could instead have continued euthermic life for the same cost as rewarming. It is seen that for a very small animal, even short periods of hibernation (torpor) are energetically meaningful, because if the time spent at low temperatures exceeds 3 h the animal has gained energetically. Daily torpor is not energetically meaningful for larger animals, because the rewarming equivalent exceeds 24 h. It may be noted that in reality, the values are conservative because the rewarming equivalent is based on metabolic rate at thermoneutrality; in reality, animals entering torpor or hibernation are in a cold situation where their metabolism would be increased to compensate heat loss (figure 2) and the rewarming equivalent thus constitutes in reality fewer hours than calculated here.)

body mass	animal	metabolic rate	rewarming cost	rewarming equivalent
kg		W	KJ	h
0.01	mouse	0.11	1.25	3
0.1	rat	0.59	12.5	6
1	—	3.34	125	10
10	marmot	18.8	1250	18
100	man	106	12500	33
1000	bear	593	125000	58

ENTRY INTO HIBERNATION

There is still no way in which we can induce an animal to hibernate, but we can now, mainly thanks to the electrophysiological work of Heller and co-workers, follow some of the early events during entry into hibernation (Heller *et al.* 1978, 1989).

From these studies, it is clear that a hibernation bout may only be initiated when the animal is asleep (in the normal sense). Neurologically, hibernation seems to be an extension of the so-called slow-wave sleep (or non-REM ('rapid-eye-movements') sleep) that occurs during the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

normal sleep state (figure 4). In the hibernation bout the awake state is totally absent, even when defined electrophysiologically, but also the quasi-awake state of REM sleep (with EEGs similar to those of awakefulness but without muscular movements) is eliminated. In all mammals, the slow-wave sleep state is characterized by a lowered set-point for body temperature (Heller & Glotzbach 1977). It would seem that what occurs in the hibernating mammal is indeed a change of this set-point to a still lower temperature. Thus the hibernating mammal still regulates its body temperature albeit at a lower level. This is clearly seen in figure 5.

No active cooling occurs during the phase of entry into hibernation and no hibernator can enter the dormant state in an environment with a temperature that approaches 37 °C.

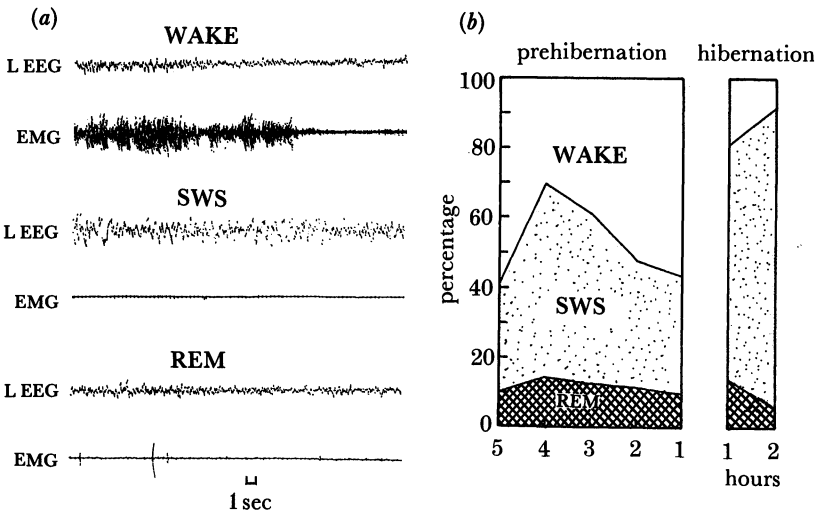


FIGURE 4. Sleep-state analysis during entry into hibernation (*a*) Typical recordings of electroencephalograms (L-EEG) and electromyograms (EMG) in ground squirrels in wakefullness, slow-wave sleep (SWS) and non-slow wave or rapid-eye movement (REM) sleep. (*b*) Relative proportions of wakefullness, SWS and REM sleep during entry into hibernation. (Adapted from Walker *et al.* (1977).)

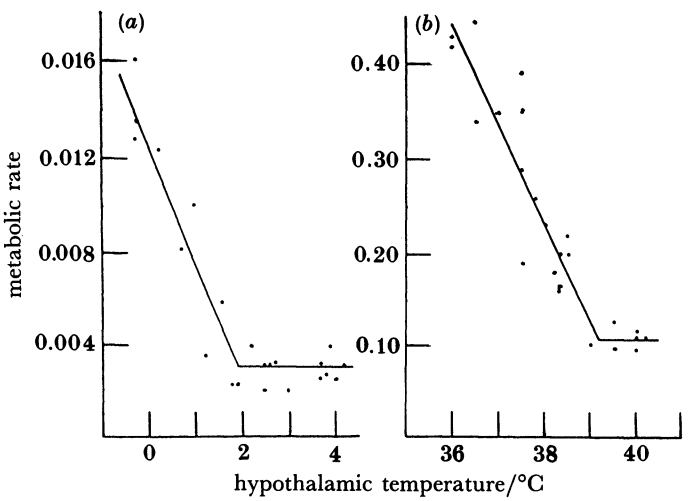


FIGURE 5. Characteristics of hypothalamic sensitivity of ground squirrels during (*a*) hibernation and (*b*) euthermia. Cooling of the preoptic anterior hypothalamus below the body temperature set point leads to an elevation of metabolic rate. This elevation occurs below 39 °C for the euthermic animal but first below 2 °C for the hibernating animal. (Adapted from Heller *et al.* (1978).)

Life at low temperature

In the hibernating hibernator we have the unusual situation of a mammal being alive and well at a body temperature below 10 °C. It could be expected that this low body temperature would lead to widespread detrimental effects on the functioning of the cells of the body. It is generally considered that mammalian enzymes have been selected for functioning at 37 °C and it could be believed that their function would become impaired and uncoordinated at lower temperatures. However, even in the normal 'homoethermic' mammal, so-called 'regional hypothermia' is common; the cells in our extremities regularly function at temperatures much below 37 °C and it is therefore not self-evident that our cell systems in general cannot continue to work at lower temperatures.

Therefore, although much discussion has taken place, the first statement must still be that most chemical processes that are balanced at 37 °C would also be in balance at a lower temperature, but they would, of course, occur more slowly. Reports of 'inhibited' metabolic processes during hibernation are often difficult to evaluate, as indeed practically all processes would be expected to proceed 30-times slower than normal – a rate generally indistinguishable from zero.

There are, however, some processes related to function at low temperatures that make them of particular interest in hibernation research, and some of these are mentioned below.

Metabolic readjustments

Just as in normal sleep, hibernation is a starvation period, and all signs of adjustment to a (semi-)starvation state occur. One difference may be that whereas carbohydrate stores are normally utilised early in a normal starvation period, these stores may be partly protected during a hibernation bout. However, this carbohydrate sparing is difficult to distinguish from the reduced rate of carbohydrate utilization that results from the depressed metabolism itself. Enzymatic adaptations also occur; for example, pyruvate dehydrogenase (the enzyme channelling carbohydrates into the citric acid cycle) becomes dephosphorylated and inactive; carbohydrates are therefore spared (Storey 1989). As pyruvate dehydrogenase phosphorylation is under insulin control (Denton *et al.* 1984), this again may simply be explained as an adaptation to starvation in which insulin levels and pyruvate dehydrogenase activity are low.

Instead, the metabolism shifts to lipid catabolism. The recorded respiratory quotients approach 0.7 (South & House 1967), indicative of lipid combustion, and the stores of lipids in the body become depleted (Nedergaard & Cannon 1984).

Ionic imbalance

One series of processes in which one would predict that problems could arise at low temperatures would be those involved in the regulation of ion balances in the cells of the body. It would be especially detrimental if the ion balances of the cells in the nervous system were destroyed during life at low temperatures.

In general, if mammalian cells are transferred to an environment with a temperature close to 0 °C, the ionic gradients normally associated with cell function gradually disappear. This is interpreted such that the processes by which the ion gradients are normally eliminated, which are expected to be mainly diffusion processes, are physical processes that have Q_{10} values close to 1; they thus continue unaffected by a reduction in temperature. However, the counteracting

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

processes, the 'ion pumps' (especially the Na^+/K^+ ATPase) are chemical processes with the 'normal' Q_{10} values of such processes of 2–3. If these two processes are approximately in balance at 37 °C, there is good theoretical reason to think that the capacity of the ion pumps would be much too small at low body temperatures, and, as stated, in experiments with mammalian cells, these predictions are generally confirmed.

Whether specific adjustments have occurred in the cells of the hibernators has especially been investigated by Willis and co-workers (Willis *et al.* 1989; Zlou & Willis 1989). These investigators have worked mainly with red blood cells as models, not because the problems of ionic balance are especially important in red blood cells but because, in work with most other cell types, it can be argued that an increased ion permeability may be artificially induced by the isolation process.

Although it has been possible to observe differences in the ionic behaviour between red blood cells from hibernators and from non-hibernators (table 3), these differences seem rather small, and a molecular understanding of the mechanisms for maintaining ionic gradients at low temperatures is still far away.

TABLE 3. A COMPARISON OF Na^+ FLUX DATA IN HIBERNATORS AND NON-HIBERNATORS[a]

(Na^+ influx and efflux were followed in red blood cells obtained from the indicated species. In all investigated species, it can be seen that the relation between influx and efflux is close to unity at normal body temperatures, but that whereas non-hibernators lose Na^+ from their red blood cells at 5 °C, hibernators apparently possess a mechanism allowing for better maintenance of ionic balance at low temperatures. Adapted from Willis *et al.* (1989).)

	Na^+ influx/Na^+ efflux	
	at 37 °C	at 5 °C
non-hibernators		
rat	0.8	2.9
grey squirrel	1.3	3.7
guinea pig	0.9	4.6
man	0.9	3.7
hibernators		
ground squirrel	1.2	1.7
hamster	0.9	1.2

Possible membrane phase transitions

For cellular membranes, as well as for all other fluid structures, there is a temperature below which the structures are in a 'frozen' state. If a transition into this rigid state should occur in the temperature interval between euthermia and hibernation, this could be expected to result in detrimental effects on cellular function.

Studies undertaken to investigate this possible phenomenon have particularly followed membrane-bound enzymatic processes in mitochondrial membranes. In such studies, 'breaks' in Arrhenius plots have been observed in mammalian non-hibernators at temperatures of about 25 °C (Lyons & Raison 1970, 1971) and these breaks have been interpreted to indicate a change from a fluid to a liquid-crystalline state in the membranes. However, in later studies in which mitochondrial membrane fluidity was examined with an electron spin resonance probe (Cannon *et al.* 1975) no evidence of any sudden change of fluidity was observed. This is principally in agreement with the very high unsaturation that the fatty acids of the mitochondrial membranes have in general (Cannon & Polnaszek 1976). In hibernators, it is possible to see changes in the apparent break point of Arrhenius plots of mitochondrial

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

membrane enzyme activities as an effect of time of year (Raison *et al.* 1988) but to what extent these breaks reflect true phase shifts in the membranes is still not known. It would, however, seem clear that no so-called 'homeoviscous adaptation' takes place in mitochondrial membranes; i.e. there is no change in fatty acid composition as an effect of cold acclimation that could explain any change in fluidity that may or may not be induced as an effect of cold acclimation (Cannon & Polnaszek 1976; Aloia & Raison 1989).

Ventricular fibrillation

From clinical and experimental evidence, the major risk factor for the non-hibernator (such as man) in severe hypothermia is, however, not related to any of the above processes but is a much more concrete problem. At body temperatures around 20 °C, especially during the rewarming process, apparently all non-hibernators develop ventricular fibrillation and die as a result of heart failure. This never happens during a hibernation and only rarely in hypothermic hibernators out-of-season. Studies on the function of the heart of the hibernator and comparisons with hearts from non-hibernators have been performed by Johansson (Duker *et al.* 1983) and by Burlington (Burlington & Darvish 1988; Burlington & Milson 1989), but again we have to conclude that, although it has been well demonstrated that differences exist between the behaviour of hearts from these two groups, the cellular and possible molecular background for these differences remains unknown.

What terminates a bout?

Although any severe disturbance, such as the experimentator's manipulation of a further reduction in environmental temperature, tends to induce arousal in the hibernator, the hibernator left to itself will regularly arouse during the hibernation season. In most hibernators, the length of the hibernation bout is only in the order of a few days, and as the winter is much longer, a series of energetically expensive arousals will occur.

The purpose of these arousals has long been discussed. Poetically one could imagine that the animals awoke to find out whether winter was yet over and spring had come. More prosaic suggestions that these arousals are necessary to eliminate waste products have frequently been put forward.

The most interesting observation concerning this problem remains that of Twente & Twente (1965) who found that the length of the hibernation bout was a function of the environmental temperature and thus of body temperature during the dormant phase. The lower the temperature, the longer was each bout, and the data could be fitted to an Arrhenius plot with a Q_{10} of nearly 3 (figure 6). Furthermore, and most interestingly, the relation could be extended to normal body temperature, and the graph then predicted a length of a 'hibernation bout' of about 8 h, i.e. the length of a normal daily sleep period. Based on this, the most appealing explanation for the cessation of each hibernation bout is that it ceases for the same reason as a night's sleep ceases (and as we do not know why we sleep or why we sleep for a specified time, the question is then eliminated from the concerns of the hibernation researches and referred to of sleep researchers).

That time is measured by the hibernating animal is indirectly demonstrated by the fact that the activity of the suprachiasmatic nucleus, which is believed to be the anatomical 'localization' of the circadian clock, is very high in the hibernating state, relative to the activity of the rest of the brain. This has been demonstrated with 2-deoxyglucose labelling studies (Kilduff &

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

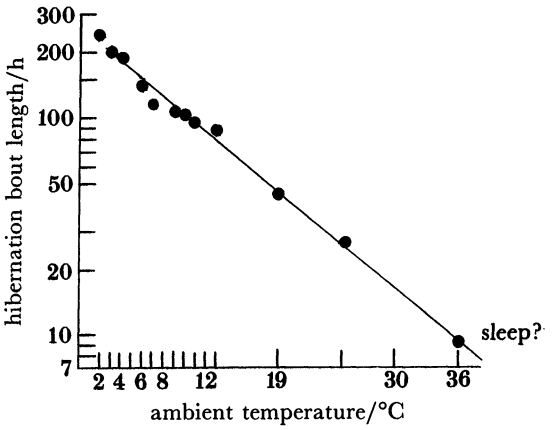


FIGURE 6. Length of hibernation bout as a function of ambient temperature. Ground squirrels were placed in the indicated environmental temperatures and the length of their hibernation bouts recorded. (Adapted from Twente & Twente (1965).)

Heller, 1989). There is an ongoing discussion as to whether the circadian clock is ticking, and at what rate, during hibernation. The studies of Twente & Twente suggest that the clock is ticking, but at a temperature-dependent rate, and that when it has ticked itself through what its body interprets as a normal sleep period, the animal wakes, although at this time, clocks of non-hibernators have advanced much more.

AROUSAL FROM HIBERNATION

The day comes when the hibernator rewarms. The most impressive point of this process is that the hibernator can rewarm itself without any help from the surroundings. Thus the popular view that the hibernators lay around waiting for the sun to rewarm them has no physiological basis. In fact, throughout the winter, the hibernator repeatedly rewarms itself within its hibernaculum.

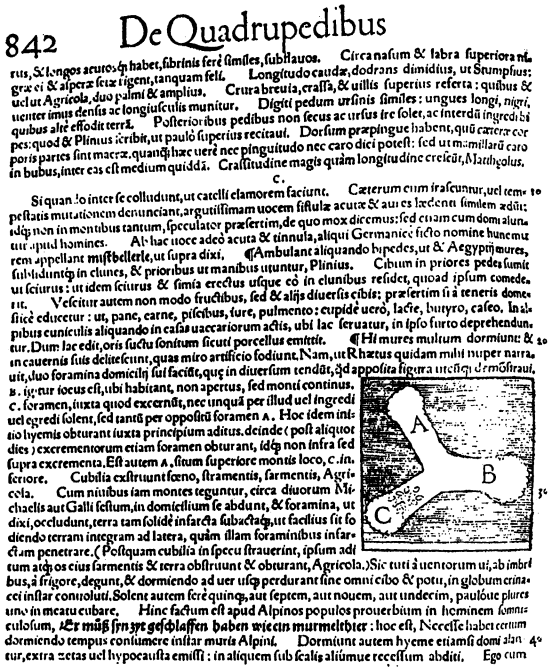
In experiments in which muscle contraction has been inhibited by curare, it has been established that an important, and in certain species the only, source of heat used for the rewarming process is that derived from processes that cannot be blocked by curare, i.e. so-called non-shivering thermogenesis (Hayward & Lyman 1967).

Until only ten years ago, the anatomical localization of the process of non-shivering thermogenesis was much debated and it was generally thought that this process mainly took place in muscle. Foster & Frydman (1978, 1979) presented convincing evidence that the organ responsible for non shivering thermogenesis was brown adipose tissue.

Brown adipose tissue

Brown adipose tissue has long attracted attention in the hibernation research area. The first known description of the tissue concerns its existence in the marmot (Gessner 1551) where it is discussed in direct relation to the hibernation behaviour of this animal (figure 7). More recently, brown adipose tissue was rediscovered under different conditions and thus became known by different terms, one being 'hibernating gland' or 'Winterschläferdrüse'; indeed the impressive article by Rasmussen (1923), in which it is clearly shown that the tissue, in its species distribution, is not restricted to hibernators, has the title 'The so-called hibernating gland'.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES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rus, & longos acuros, qh habet, fibrinis ferè similes, subflauos. Circa nasum & labra superiora ni-
grae ei & alperæ setæ tigent, tanquam felt. Longitudo caudæ, dodrans dimidius, ut Stumphus:
uel ut Agricola, duo palmi & amplius. Crura breuia, crassa, & uillis superius referta: quibus &
uenter imus densis ac longiusculis munitur. Digiti pedum ursinis similes: ungues longi, nigri,
quibus altè effodit terrã. Posterioribus pedibus non secus ac ursus ire solet, ac interdũ ingredi bi
pes: quod & Plinius scribit, ut paulò superius recitaui. Dorsum præpingue habent, qũ cæteræ cor
poris partes sint macræ, quanqh hæc uerè nec pinguitudo nec caro dici potest: sed ut mamillarũ caro
in bubus, inter eas est medium quiddã. Crassitudine magis quàm longitudine crescit, Matthæolus.

c.

Si quando inter se colludunt, ut catelli clamorem faciunt. Cæterum cum irascuntur, uel tem-
pestatis mutationem denunciant, argutissimam uocem fistulæ acutæ & auribus lædenti similem edũt:
idqh non in montibus tantum, speculator præsertim, de quo mox dicemus: sed etiam cum domi alun-
tur apud homines. Ab hac uoce adeò acuta & tinnula, aliqui Germanicè ficto nomine hunc mu-
rem appellant **mistbellerle**, ut supra dixi. ¶Ambulant aliquando bipedes, ut & Aegyptij mures,
subsidunth in clunes, & prioribus ut manibus utuntur, Plinius. Cibum in priores pedes sumit
ut sciurus: ut idem sciurus & simia erectus usque eò in clunibus residet, quoad ipsum comede-
rit. Vescitur autem non modo fructibus, sed & alijs diuersis cibis: præsertim si à teneris dome-
sticè educetur: ut, pane, carne, piscibus, iure, pulmento: cupidè uerò, lacte, butyro, caseo. In ali-
pibus cuniculis aliquando in casas uaccariorum actis, ubi lac seruatur, in ipso furto deprehendun-
tur. Dum hæc edit, oris fuctu sonitum sicuti porcellus emittit. ¶Hi mures multum dormiunt &
in cauernis suis delitescunt, quas miro artificio fodiunt. Nam, ut Rhætus quidam mihi nuper narra-
uit, duo foramina domicilij sui facit, quę in diuersum tendũt, ãd appolita figura uti hic demõstrat.
B. iacet locus est, ubi habitant, non apertus, sed monti continus.
c. foramen, iuxta quod excernũt, nec unquã per illud uel ingredi
uel egredi solent, sed tantũ per oppositũ foramen A. Hoc idem ini-
tio hyemis obturant iuxta principium aditus. deinde (post aliquot
dies) excrementorum etiam foramen obturant, idqh non infra sed
supra excrementa. Est autem A, situm superiore montis loco, c in-
feriore. Cubilia exstruunt fœno, stramentis, sarmentis, Agri-
cola. Cum niuibus iam montes teguntur, circa diuorum Mi-
chaelis aut Galli festum, in domicilium se abdunt, & foramina, ut
dixi, occludunt, terra tam solidè infarcta subactaqh, ut facilius sit fo
diendo terram integram ad latera, quàm illam foraminibus infar-
ctam penetrare. (Postquam cubilia in specu strauerint, ipsum adi
tum atqh os eius sarmentis & terra obstruunt & obturant, Agricola.) Sic tuti à uentorum ui, ab imbri
bus, à frigore, degunt, & dormiendo ad uer usqh perdurant sine omni cibo & potu, in globum crina-
cei instar conuoluti. Solent autem ferè quinqh, aut septem, aut nouem, aut undecim, paulòue plures
uno in meatu cubare. Hinc factum est apud Alpinos populos prouerbium in hominem somno-
culosum, **Er muß syn yre geschlaffen haben wie ein murmeltyer**: hoc est, Necesse habet eum
dormiendo tempus consumere instar muris Alpini. Dormiunt autem hyeme etiamsi domi alan-
tur, extra aetas uel hypocausta emissi: in aliquem sub scalis aliúmue recessum abditi. Ego cum

FIGURE 7. The first association of brown adipose tissue with hibernation. A copy of page 842 in Gessner (1551). The description of brown fat in marmots is found on lines 6–8; 'Although other body parts are meagre, they (i.e., the marmots) have a very fat back, but this (tissue) can really neither be called fat nor flesh: rather it is something between these (two), just as is the case with the udder of the cows. According to Mattheolus, they (i.e. the marmots) rather grow in thickness than in length'. In the following, their winter sleep is described, and Gessner wonders how they can get the air for respiration in their tightly sealed burrows. (Search and translation by T. Christiansen and J. Öberg.)

However, the function of the tissue in hibernation remained obscure. But, as is evident from the names used, it was generally believed that it had a glandular function and that it secreted some factor necessary for hibernation. (So-called 'hibernation trigger' substances are still discussed in hibernation research (Oeltgen *et al.* 1989) but they have proven to be elusive and their existence and necessity are presently doubted (Wang *et al.* 1988; Wang & Lee 1989)).

It was not until Smith & Hock (1963) and Smalley & Dryer (1963) showed that brown adipose tissue is the warmest organ of the body during arousal that it was realized that the function of brown adipose tissue in the hibernator is not hibernation but rather the reverse: to produce the heat necessary for arousal.

The function of brown adipose

In figure 8, we have summarized the function of the brown fat cell (Nedergaard & Lindberg 1982). When the cell is stimulated via the sympathetic nervous system, fat, stored in the cell, is broken down to free fatty acids. These fatty acids may either be exported from the cell (Nedergaard & Lindberg 1979; Nedergaard 1982) or be combusted within the brown-fat cell itself. In contrast to all other cell types, the oxidation of substrates (such as fatty acids) in the brown-fat cell is not necessarily coupled to the production of ATP. The ATP synthesis pathway can be circumvented because of the existence in the brown-fat cell mitochondria of a unique protein, the uncoupling protein, thermogenin (for reviews, see Nicholls & Locke 1984; Cannon & Nedergaard 1985). Thermogenin activity is the rate-limiting step in non-shivering

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

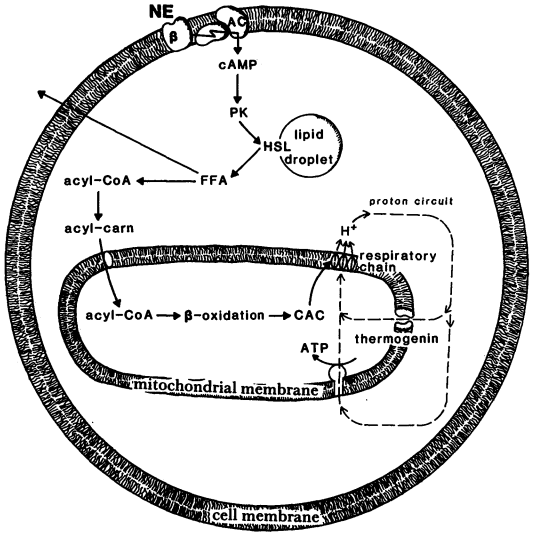

FIGURE 8. The function of brown adipose tissue. Norepinephrine (NE) released from the sympathetic nervous system innervating the tissue binds to β-receptors and activates adenylate cyclase (AC), which leads to an increase in the intracellular concentration of cyclic AMP (cAMP). This activates a protein kinase (PK), which phosphorylates and thereby activates hormone-sensitive lipase (HSL). This lipase breaks down triglycerides stored in the cell; the released free fatty acids (FFA) may either be released from the cell into the circulation or may be oxidized in the cell. For this latter purpose, the fatty acids are activated to acyl-CoA derivatives, which (as acyl carnitines) enter the mitochondria where they are broken down via so-called β-oxidation and the citric acid cyclic (CAC) and thus combusted to CO_2 and water. It is likely that it is the free fatty acids themselves or their acyl-CoA derivatives that interact with the uncoupling protein thermogenin, found exclusively in the mitochondrial inner membrane of brown adipose tissue. When thermogenin is activated, the proton circuit of the mitochondria is short-circuited, and the electrochemical energy, which otherwise would be used to synthesize ATP, is directly dissipated as heat. (Adapted from Cannon *et al.* (1988).) For further details see Nedergaard and Lindberg (1982); Cannon & Nedergaard (1985).

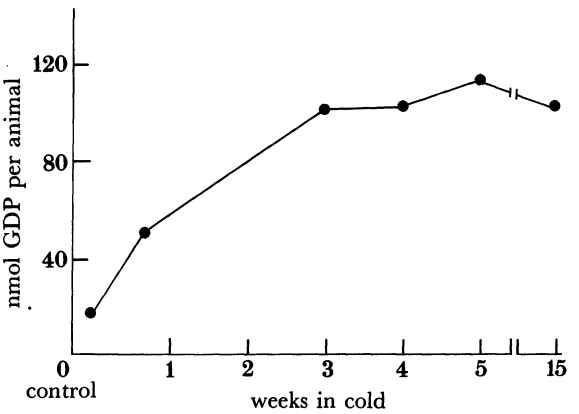

FIGURE 9. Changes in thermogenin content of a hibernator after cold exposure. Amount of thermogenin is here estimated in isolated brown-fat mitochondria from golden hamsters by the so-called GDP-binding method (for details, see Sundin *et al.* (1987).)

thermogenesis (Cannon *et al.*, 1981). Therefore, there is good reason to assume that the presence of an adequate amount of thermogenin is a prerequisite for successful arousal.

In figure 9 we have followed the amount of thermogenin during the adaptive phase before a hibernation period; it is seen that a substantial increase in the amount of this protein occurs before the onset of hibernation (Sundin *et al.* 1987). However, during the hibernating phase

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of a single hibernation bout, the activity of thermogenin is expected to be very low, as no heat production is expected during this time. Horwitz *et al.* (1985) and Nedergaard *et al.* (1989) observed that thermogenin is in what has been dubbed a 'masked' form that, at least experimentally, has been associated with an inactive state of the protein.

However, there is good reason to assume that brown-adipose tissue plays a major role in producing the heat necessary for the arousal process. Indirect evidence for this can be obtained from following what happens with the fatty acid stores in the tissue during a hibernation bout (figure 10) (Nedergaard & Cannon, 1984; Carneheim *et al.* 1989). Apparently, during entry into hibernation (when the tissue is producing no heat) there is an accumulation of lipid in the tissue. Although most of this lipid comes directly from the circulating lipids, which in their turn come from dietary fat, a surprisingly large fraction of the lipid accumulated may, at least under experimental conditions, be the rare fatty acid homo-gamma-linolenic acid (20:3). When arousal is initiated, the lipid stored in the brown fat disappears, preferentially the rare fatty acids (whereas nothing happens during this phase to the lipid found in white adipose tissue).

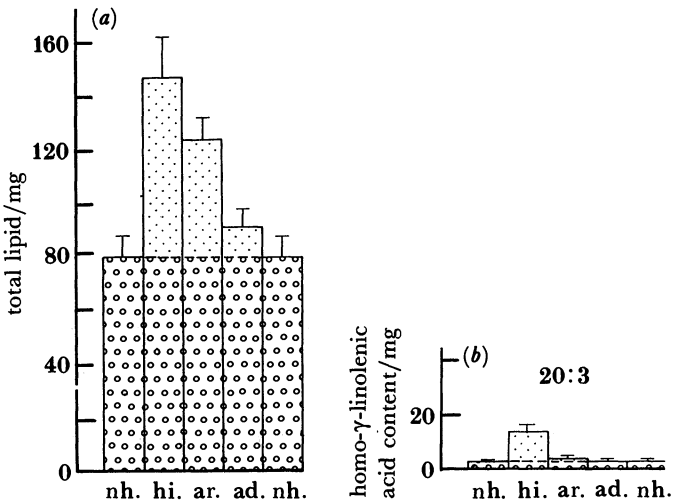

FIGURE 10. Dynamics of lipid turnover in brown fat during a hibernation bout in the golden hamster, (*a*) Total amount of lipid in interscapular brown adipose tissue. (*b*) changes in the content of homo-γ-linolenic acid; nh., non-hibernating, cold-acclimated animals; hi., animals in deep hibernation; ar., animals in the middle of the arousal phase; a.d, animals fully aroused from hibernation. (Adapted from Nedergaard & Cannon (1984) and Carneheim *et al.* (1989).)

In a golden hamster, the total amount of heat that would be evolved from the combustion of the amount of lipid which disappears from brown adipose tissue amounts to 10 kJ. The total amount of heat needed to rewarm a hibernating hamster amounts, theoretically, to 8 kJ; it has been measured by Robertson *et al.* (1968) to be 11 kJ. Thus the heat released from the combustion of the fat disappearing from brown adipose tissue is sufficient to rewarm the entire hamster.

However, even though brown-fat cells are very efficient heat producers, they also have to obey the laws of chemical processes, and the Q_{10} for their heat production is not different from that of other cell types (Nedergaard *et al.* 1977). This may be the reason that in most hibernators, non-shivering thermogenesis does not produce sufficient heat for the arousal process, and it may be that during the arousal process, brown adipose tissue produces some of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

'its' heat by releasing fatty acids for combustion during shivering thermogenesis in skeletal muscle (Cannon *et al.* 1978).

Preparation for hibernation

As understood from the above, the process of hibernation is seasonal. Thus, for a hibernator, there is a time of the year in which it is prone to enter hibernation and there are other times in which it does not seem possible to invoke this behaviour. We have already noted the necessity of a preparatory phase for the development of an adequate amount of brown adipose tissue.

Even though hibernators do not have the need for the immense preaccumulation of food that is necessary for the non-hibernator to survive winter, it is still necessary for the hibernator to accumulate sufficient energy to sustain their metabolism (albeit low) and to fuel the rewarming during arousals. This accumulation may take place in the form of hoarding (as in smaller hibernators such as the hamster) but it often occurs in the form of fattening of the hibernator before winter. Thus, in many hibernators, appetite is regulated in an annual pattern, and food intake and body mass may change dramatically during the year (Pengelley 1967). This seasonal accumulation of body lipids ('voluntary obesity') makes 'fattening hibernators' an interesting but little exploited model in obesity research. The accumulation of fat in the preparatory season also presents interesting regulatory problems when it is remembered that this occurs concurrently with brown adipose tissue recruitment. As recruited brown adipose tissue normally counteracts an increased fat accumulation (Rothwell & Stock 1979), we seem to have a situation in the preparatory hibernator in which brown adipose tissue can be recruited without concomitantly being active; this is quite different from what has been understood from studies of brown fat recruitment in general (during cold acclimation) but may be reminiscent of what happens during brown-fat recruitment *in-utero* in certain species (Cannon *et al.* 1988).

It is believed that other changes also occur during the preparatory phase, and it is at least temporarily, if not functionally, associated with an involution of the gonads. Also, a change in higher brain function must take place, allowing the animal to 'decide' that one day it will no longer fight the cold but instead allow itself to sink into slow-wave sleep. Indeed, it has been proposed that some human winter depressions may have similarities with the central nervous system changes that are encountered in the preparatory hibernator (Wehr 1989). In both circumstances, these changes may be evoked largely by the decreasing intensity and duration of the light during autumn.

To hibernate or not?

In a patiently performed study, Lyman *et al.* (1987) investigated the lifespan of hibernating and non-hibernating hamsters. They found that hibernation apparently prolonged life expectancy: for poor hibernators, life expectancy was only 727 days; for good hibernators it was 1093 days. However, many of these days were spent in dormancy, but even if we only count euthermic days, the poor hibernators were 'alive' for 687 days and the good hibernators for 809 days. Thus the hibernators actually gained more active days. However, in hamsters that were never in the cold, life expectancy was as much as 812 days. Thus it would seem that the best way to prolong 'active' life is to stay away from the cold.

To 'prolong' life further in biological terms means to be able to produce ample numbers of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

descendants. It is therefore noteworthy that a hibernating hamster, because it survives for several hundred calendar days more than its non-hibernating kin, may live to experience a third spring and may thus be a more successful ancestor.

Thus, for those mammals that have the ability, hibernation may be a very good choice. Sometimes, when winter darkness veils the sky, the thought may not be far away that, given the choice, even mankind may decide to spend the winter in a quiet hibernaculum and to recolonize the earth when the birds of spring reappear.

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Discussion

R. Jaenicke (*Institut für Biophysik und physikalische Biochemie, Universität Regensburg, F.R.G.*). Is the early phase of hibernation connected with the well-defined cold inactivation – cold dissociation processes at the level of key enzymes of metabolism?

Barbara Cannon. As the entry phase of hibernation commences at normal high body temperature, the reduction in metabolic rate is not in itself caused by any cold inactivation as such, but should be seen as a controlled inhibition of metabolic–thermogenic processes. To my knowledge, there are no reports of cold dissociation phenomena occurring during entry into hibernation.

R. Jaenicke. Is thermogenin a common uncoupler? Could Professor Cannon comment on its biochemical and physical properties? Do adults that seem to have negligible amounts of brown fat still express thermogenin?

Barbara Cannon. The uncoupling protein, thermogenin functions in principle as a common

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

uncoupler in that it acts as a protonophore. Its sequence is known both from amino-acid analysis and from sequencing of several cDNA clones, which are now available. The monomeric molecular mass is about 32–33 kDa in the different species analysed. It probably exists as a dimer in the mitochondrial inner membrane. The monomer is suggested to have a tripartite structure and to span the membrane six times. The nucleotide binding site is on the external face. From an evolutional viewpoint, the protein is believed to derive from the mitochondrial adenine nucleotide translocase and perhaps more distantly from the mitochondrial phosphate transporter. The potential ability to be able to express the thermogenin gene is believed still to be present in adult humans, although the extent to which this ability is normally utilized is poorly understood. Under pathological circumstances, such as phacochromocytoma, the gene is highly expressed, presumably because of the massively enhanced sympathetic drive.

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