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SEASONAL AND TEMPERATURE-RELATED CHANGES IN MITOCHONDRIAL MEMBRANES ASSOCIATED WITH TORPOR IN THE MAMMALIAN HIBERNATOR *SPERMOPHILUS RICHARDSONII*

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Seasonal variations in the thermal response of liver mitochondrial membranes from Richardson's ground squirrels *(Spermophilus richardsonii)* were determined by measuring succinate-cytochrome *c* reductase activity and spin label motion over a temperature range of 2°C to 35°C. For seven summer animals from the field the Arrhenius-type plots for enzyme activity and spin label motion were biphasic indicating a transition in structure and function at 22 + 2.3°C and 23 ± 1.9°C, respectively; typical of homeothermic mammals. For 12 winter animals maintained at 19°C, the transition in structure and function was lowered to 12 ± 1.1°C and 13 ± 1.4°C, respectively. The transition for 5 of 11 winter animals which were kept at 4°C and maintained normal activity and body temperature was similar to animals maintained at 19°C, while for the other six the transition was further lowered to less than 4°C. The transition for seven winter animals which were in deep hibernation was less than 4°C. The results for liver mitochondria show that lowering of the transition in membrane structure and function occurs as a two-stage process of about 10 deg. C for each stage and that the lowering is a requisite for hibernation rather than a response to the low-body temperatures experienced during hibernation.

Introduction

Temperature-related membrane phenomena often display an abrupt transition at a defined temperature, $T^{\star}$. For membrane-associated enzymes, this $T^{\star}$ is the temperature at which there is an abrupt change in the apparent Arrhenius activation energy (E_a). Such changes have been shown with liver mitochondrial membranes from endotherms (animals whose body heat is largely supplied by metabolism) for succinate oxidase [1,2], cytochrome *c* oxidase [3,4] and cytochrome *c* reductase [4,5].

The thermal response of similar membrane-associated enzymes from ectotherms (animals whose body heat comes from the environment) are much less predictable. Linear Arrhenius-type plots, i.e. those in which E_a does not change over a temperature range of about 4° to 30°C, have been reported for succinate oxidase from liver of toads [6], trout [7,8], catfish [7] and frog skeletal muscle [9]. However, a biphasic plot was reported for mitochondria from frog muscle oxidising glutamate [9]. Furthermore, liver mitochondria from carp showed an adrupt change in E_a for both succinate and cytochrome *c* oxidase and the $T^{\star}$ for these enzymes changed with acclimation temperature [10]. In contrast, the $T^{\star}$ for succinate

oxidation of goldfish liver, at 20 °C, did not shift with acclimation [11].

For some animals, the temperature at which the E_a of a membrane-associated enzyme changes appears to be related to the thermal history (acclimation) and thermal strategy. Shifts in $T^\star$ have been shown in ectotherms with acclimation, but in most endotherms $T^\star$ remains constant, usually between 20 ° and 25 °C. However values for $T^\star$ as low as 15 °C have been reported for state III respiration of mouse liver mitochondria [1], for young rats fed a diet rich in unsaturated fatty acids [2], and for thyroidectomized rats [12]. This suggests that $T^\star$ is not invariant, even in homeothermic endotherms, although for these animals $T^\star$ is approximately the minimal experimental temperature at which vital functions such as heart beat can continue [13–15]. It should be stressed that not all endotherms are homeothermic, and the most studied heterothermic endotherms are the mammalian hibernators. Raison and Lyons [16] found that $T^\star$ for succinate oxidase activity from liver mitochondria of active ground squirrels (*Citellus lateralis*) was 21 ° to 25 °C, typical of a mammal, but for the same enzyme from torpid ground squirrels the E_a was constant over the temperature range of 4 ° to 40 °C, indicating a $T^\star$ below 4 °C.

The E_a of an enzyme catalysed reaction can change for a variety of reasons and an increase at low temperature is not an exclusive property of membrane-associated enzymes [17]. It has been suggested, however, that the abrupt change in E_a for the oxidative enzymes of mitochondrial membranes is associated with, and results from, a thermotropic alteration in the ordering of some membrane lipids [18,19]. The methods used to detect these changes in ordering usually depend on measuring the motion of probes intercalated with the hydrophobic domain of membrane lipids or liposomes formed from membrane lipids. While there is some debate about the relevance of the absolute values of motion and fluidity obtained using these methods, they provide useful comparative information of the thermal response of membrane lipids. With mitochondrial membranes, changes in the temperature coefficient of probe motion have been detected at temperatures which coincide with the $T^\star$ obtained for the Ea of enzymes associated with these organelles [19,20]. This coincidence is maintained even when $T^\star$ is shifted by experimental techniques such as diet adjustment [2] or thyroidectomy [12]. Changes in the temperature coefficient of spin label motion are not detected in torpid endotherms [20–22] over the range of temperatures which give linear Arrhenius plots for membrane-associated enzyme activity.

Differences in the thermal response of both the membrane-associated enzymes and the membrane lipids observed with torpid hibernators, compared with active individuals of the same species, might result from acclimation of the membrane to the low body temperature of torpor as observed in some ectotherms [10]. However, two studies have shown that alterations in the thermal response of membrane lipids occur in heterothermic endotherms in their natural torpor season independently of a lowering of body temperature. Augee et al. [23] found the $T^\star$ for blood cell lipids of hedgehogs shifted from about 19 °C in summer to about 14 °C in winter even though the animals remained homeothermic throughout the year in a laboratory colony under relatively constant temperature. Similarly, with hamsters, Cremel et al. [24] found the $T^\star$ for succinate induced intramitochondrial protein release varied from a maximum value of 29 °C in spring to a minimum value of 14 °C at the end of summer. These results strongly suggest that the thermal-induced changes in mitochondrial membrane structure and function observed in torpid heterotherms are not entirely due to acclimation or 'homeoviscous adaptation' as has been described for ectotherms [25] but are, at least in part, achieved by some cyclic physiological processes that may prepare the animal for the low body temperatures of winter torpor.

We have examined various experimental groups and natural populations of the mammalian hibernator, Richardson's ground squirrel, *Spermophilus richardsonii*, in order to investigate the relationship between the $T^\star$ for the structural and functional properties of liver mitochondrial membranes and the annual cycles, ambient temperature and physiological state of the animal.

Materials and Methods

Richardson's ground squirrels, *Sphermophilus richardsonii*, were trapped in the vicinity of

Edmonton, Alberta. Laboratory animals were fed ad libitum on a diet of pelleted laboratory chow (Vitamite cubes consisting of 25% protein, 5% fat and 6% fibre) supplemented with unsalted sunflower seeds, and maintained in individual cages at a controlled temperature of 19 ± 1°C with a 12/12 h, light/dark photoperiod, except for cold-exposure groups which were held at 4°C without food, with a photoperiod of 2/22 h, light/dark.

Animals were killed by decapitation and liver mitochondria isolated as described by Pehowich and Wang [26]. Enzyme assays were complete within 6 h.

Cytochrome *c* reductase was assayed as described by McMurchie et al. [5] with the temperature of the reaction mixture in the cuvette measured directly by a thermocouple probe.

For the extraction of lipids, mitochondrial pellets of about 0.1 ml packed volume were boiled for 10 min (82°C) in 4 ml of isopropanol containing 1 mg butylated hydroxytoluene per 100 ml. Following filtration, the residue was homogenized in about 4 ml of chloroform/methanol (2 : 1, v : v). The resulting suspension was filtered and the precipitate washed twice with chloroform/methanol (2 : 1, v : v). Traces of protein were removed by treatment with Sephadex as described by Williams and Merrilees [27]. The supernatant was dried at 35°C under reduced pressure and the residue dissolved in 4 ml of chloroform/methanol (2 : 1, v : v), to which was added 1 ml of H_2O followed by 1 g of Sephadex (G-25). The mixture was evaporated to dryness, resuspended in chloroform, re-evaporated and resuspended in chloroform. This suspension was placed in a column and the Sephadex washed twice with chloroform. The lipids were dried and stored under dry nitrogen in sealed ampoules.

In preparation for electron spin resonance spectroscopy, 5 mg samples of the above lipids were dispersed by gentle sonication in 0.5 ml 0.1 M Tris-acetate buffer (pH 7.2) containing 5 mM EDTA and 20% (v/v) ethylene glycol. Portions (0.1 ml) of this dispersion were added to approx. 0.02 μmol of the spin label 9NSM (3-oxazolidinyloxy-2[7-carbmethoxyseptyl]-2-nonyl-4,4 dimethyl) that had been deposited by evaporation onto the surface of a small glass vial. The spin label and lipid dispersion were warmed to about 40°C and mixed. The motion parameter τ_0 was determined as described by Mehlhorn et al. [28] from the first derivative spectra obtained at various temperatures within the range 2–40°C. The temperature was measured directly with a thermocouple probe placed about 0.5 cm above the cavity but in contact with the sample.

Plots of the logarithm of label motion and enzyme activity as a function of the reciprocal of absolute temperature were analysed by the method of Pollard [29] to determine the best single or two-line fit to the data. The significance of a difference in slope (m) and intercept (b) for two straight lines defined by the general equation $y = mx + b$ was tested and unless otherwise stated was significant at $P < 0.01$ based on the t distribution for n minus 5 degrees of freedom where n = total number of points for the two straight lines.

Results

For the data shown in Figs. 1, 2 and 3 it has been assumed that the E_a is linearly related to the reciprocal of the absolute temperature and the line, or lines, of best fit determined as described in the Materials and Method section. For biphasic plots the temperature at the intersection of the two linear portions of the graph was considered to be the temperature for the transition in function. There has been considerable discussion regarding the signficicance of abrupt changes in the slope of Arrhenuis-type plots and the interpretation of these changes in terms of their being representative of abrupt transitions in thermodynamic properties has been questioned. In the light of this discussion Klein [30] recently proposed a curvilinear analysis of Arrhenius plots which included parameters for the temperature of the mid-point of the transition as well as the temperature width of the transition. The data presented in this paper for enzyme activity have also been analysed by this curvilinear method using equation 59 of Klein. Data obtained from the thermal response of the motion parameter τ_0 of the spin label intercalated with membrane lipids were analysed in terms of a linear relationship between the logarithm of the motion parameter (τ_0) and the reciprocal of the absolute temperature.

For animals taken from the field in summer the

plots of both succinate-cytochrome *c* reductase activity and spin label motion were biphasic, as shown in Fig. 1, with the transition in both function (Fig. 1A) and structure (Fig. 1B) at 23° and 22°C, respectively. Fig. 2 is representative of the data from animals killed during torpor and is in contrast with that of summer animals in that no transition is apparent, at least above 4°C. Fig. 3 is representative of winter animals maintained in the laboratory at an ambient temperature T_a of 19°C. For these animals the plots are biphasic with the $T^\star$ at 13°C and 12°C, 10 deg. C lower than the corresponding $T^\star$ for function and structure of summer animals shown in Fig. 1. These data clearly demonstrate the coincidence between the $T^\star$ for structure, as indicated by the change in temperature coefficient of spin label motion, and function, as measured by the change in the E_a of succinate-cytochrome *c* reductase activity. $T^\star$ for all of the animals, in the various experimental and seasonal groups, are detailed in Tables I to IV.

The mean $T^\star$ of succinate-cytochrome *c* reductase activity for 6 of the 9 animals from the field in summer (Table I) was 22 ± 2.3°C; coincident with the 23 ± 1.9°C obtained for $T^\star$ for the temperature coefficient of spin label motion. The $T^\star$ for both the structural and functional parameters for two of the animals in this group was 13°C, about 10 deg. C lower than the other six animals (Table I). For these two animals the $T^\star$ is the same as that of animals in winter shown in Table II.

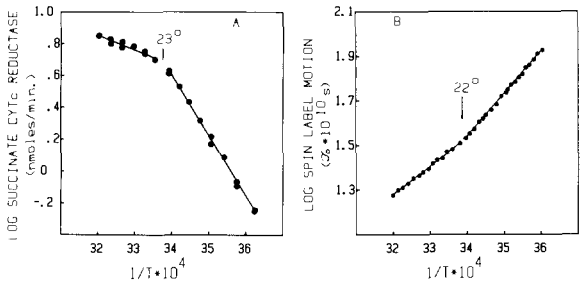

Fig. 1 Arrhenius-type plots of (A) succinate-cytochrome *c* reductase activity and (B) spin label (9NSM) motion with lipids from liver mitochondrial membranes of summer active, Richardson's ground squirrels. The $T^\star$ is indicated by the arrow and was determined by linear regression analyses as described in Materials and Methods. The motion τ_0 was calculated from the first derivative absorption spectrum at each of the temperatures shown.

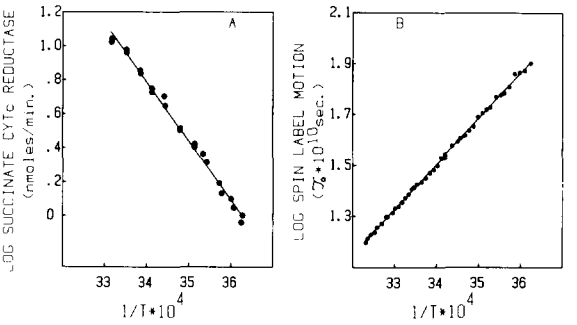

Fig. 2 Arrhenius-type plots of (A) succinate-cytochrome *c* reductase activity and (B) spin label motion associated with mitochondria from Richardson's ground squirrels in torpor. For explanation of data see Fig. 1.

The animals shown in Table II were maintained in the laboratory colony at a constant T_a of 19°C with food ad libitum and were killed after the time when all the ground squirrels had disappeared from the field in November. This group of animals remained homeothermic, and the mean $T^\star$ was 13 ± 1.1°C ($n = 12$) and 13 ± 1.4°C ($n = 11$) for the structural and functional transitions, respectively. The range for $T^\star$ was 11°C to 15°C, significantly below the range of summer field animals in Table I. Another group of animals which was maintained in the laboratory at low temperature (4°C) also remained homeothermic (Table III). The $T^\star$ for these animals fell into two distinct sub-groups. One sub-group (cf. N1, N2,

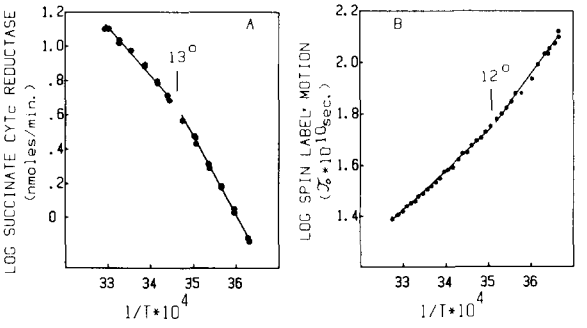

Fig. 3. Arrhenius-type plots of (A) succinate-cytochrome *c* reductase activity and (B) spin label motion associated with mitochondria from Richardson's ground squirrels maintained during winter in a laboratory colony at T_a of 19°C. For explanation of data see Fig. 1.

TABLE I

RICHARDSON'S GROUND SQUIRRELS CAPTURED AND ASSAYED IN SUMMER

(–) indicates assays were not carried out. T^* is the temperature at which a discontinuity or transition appeared in a plot ($P < 0.01$) of spin label motion (ESR) or cytochrome *c* reductase activity (Cyt *c*).

Animal number	Sex	Body wt. (g)	Date caught	Date killed	T^* (°C)	
					ESR	Cyt *c*
J2	M	430	27 Jul	28 Jul	25	25
J3	M	330	27 Jul	30 Jul	23	22
J6	M	351	27 Jul	10 Aug	–	19
J7	M	360	27 Jul	12 Aug	24	24
J8	M	411	27 Jul	26 Aug	23	–
S1	M	474	18 Sep	22 Sep	20	20
S2	M	430	18 Sep	24 Sep	21	21
				Mean	23	22
				S.D.	1.9	2.3
J4	F	233	27 Jul	4 Aug	13	14
J5	M	381	27 Jul	6 Aug	13	12

N8 to N11 in table III) had a T^* below 4°C indistinguishable from animals in torpor (cf. Table IV). The other sub-group had a mean T^* of 12.5 $\pm$ 1.3°C ($n = 10$) resembling the T^* for the homeothermic winter group exposed to 19°C (cf. Table II). For animals killed in torpor (Table IV) no change in the E_a for enzyme activity or the temperature coefficient of spin label motion was detected over the temperature range of 2 to 38°C. The T^* for these animals was therefore considered to be less than 4°C.

When the temperature dependence of the succinate-cytochrome *c* reductase is expressed as an Arrhenius plot, the slope of the line is propor-

TABLE II

RICHARDSON'S GROUND SQUIRRELS MAINTAINED AT CONSTANT TEMPERATURE (19°C) AND KILLED DURING WINTER

For explanation see legend to Table I.

Animal number	Sex	Body wt. (g)	Date killed	T^* (°C)	
				ESR	Cyt *c*
L1	F	375	25 Nov	13	15
L2	F	441	27 Nov	14	12
L3	M	444	1 Dec	14	14
L4	F	494	3 Dec	13	–
L5	F	408	16 Dec	12	12
H1	F	615	4 Nov	12	12
H2	F	559	10 Nov	12	13
H3	M	590	12 Nov	15	15
H4	F	535	16 Nov	12	12
H5	M	614	18 Nov	11	12
H6	M	538	20 Nov	12	12
H7	F	541	23 Nov	13	15
			Mean	13	13
			S.D.	1.1	1.4

TABLE III

RICHARDSON'S GROUND SQUIRRELS EXPOSED TO LOW TEMPERATURE (T_a 4°C) BUT REMAINING HOMEOTHERMIC

Killed during winter. For explanation see legend to Table I.

Animal number	Sex	Body wt. (g)	Date killed	T^* (°C)	
				ESR	Cyt *c*
N1	F	513	13 Oct	< 4	< 4
N2	M	577	22 Oct	< 4	< 4
N3	F	464	27 Oct	13	13
N4	M	538	29 Oct	12	12
N5	M	346	2 Nov	13	15
N6	M	502	6 Nov	13 [a]	12
N7	M	452	10 Dec	10	12
N8	M	494	11 Dec	< 4	< 4
N9	M	593	14 Dec	< 4	< 4
N10	M	445	15 Dec	< 4	< 4
N11	M	457	17 Dec	< 4	< 4

[a] Slope differences significant at $P < 0.5$.

TABLE IV

RICHARDSON'S GROUND SQUIRRELS KILLED IN TORPOR

For explanation see legend to Table I.

Animal number	Sex	Body wt. (g)	Date killed	Body temp.	$T^{\star}$ (°C)	
					ESR	Cyt *c*
A2	M	478	20 Aug	5.0	< 4	< 4
A3	M	426	25 Aug	5.1	< 4	< 4
A4	M	450	27 Aug	4.5	< 4	< 4
A5	M	498	1 Sep	4.0	< 4	< 4
A6	M	550	4 Sep	4.5	< 4	< 4
A8	F	316	30 Sep	4.5	–	< 4
A13	F	225	8 Dec	4.8	< 4	< 4

tional to the experimental enthalpy or apparent Arrhenius energy of activation (E_a) for the reaction. Without consideration of the molecularity of the reaction, the enthalpy has little thermodynamic significance, but it does provide a useful basis for comparisons of the temperature response of enzymes since the E_a in the temperature range of 25 to 35 °C is directly related to the temperature coefficient Q_{10}. Table V shows the means for the E_a above $T^{\star}$ for each group of animals. There was a significant increase in E_a when $T^{\star}$ was reduced in winter and a further increase when $T^{\star}$ was reduced during torpor. This relationship between E_a and $T^{\star}$ has been observed previously with succinate oxidase activity [2].

The data described in Tables I to IV have been fitted to the curvilinear equation described by Klein [30]. This type of analysis also provides an estimate of the mid-point of the transition in function, T_m. Values for T_m determined by this method of analysis are shown in Table VI and are not significantly different from $T^{\star}$ determined by the linear fit given in Tables I–IV.

The spin label used in this study is a derivative

TABLE V

THE RELATIONSHIP BETWEEN THE E_a FOR SUCCINATE-CYTOCHROME *c* REDUCTASE ABOVE $T^{\star}$ FOR EACH OF THE GROUPS OF ANIMALS DESCRIBED IN TABLES I TO IV

The E_a or experimental enthalpy was calculated from the slope of the line of best fit of the Arrhenius-type plot using the relationship E_a = slope · R · 2.303 where R is the universal gas constant in joules. All values ±1 S.D.

Experimental group	Source of data (Table)	*n*	$T^{\star}$ (°C) (from Tables I–IV)	E_a above $T^{\star}$ (kJ/mol)
Summer field active, (excluding J4,J5)	I	6	22.0 ± 2.3	28.7 ± 6.6
Winter active, T_a 19 °C	II	11	13.0 ± 1.4	54.7 ± 7.2
Winter active, T_a 4 °C (N3 to N7)	III	5	12.8 ± 1.3	56.2 ± 2.8
Winter active, T_a 4 °C (N1,N2,N8–N11)	III	6	< 4	57.3 ± 12.4
Winter torpid, T_a 4 °C	IV	7	< 4	58.8 ± 4.9

TABLE VI

COMPARISON OF TWO ANALYTIC TREATMENTS OF CYTOCHROME *c* REDUCTASE THERMAL RESPONSE DATA

T_m = mean ± S.D. of the mid-point of the transition temperatures determined by the curvilinear analysis of Klein [30]. T^* = mean ± S.D. of transition temperatures in Tables I–IV determined by the linear analysis of Pollard [29].

Experimental group	Source of data (Table)	*n*	T^* (°C)	T_m (°C)
Summer field active, (excluding J4,J5)	I	6	22.0 ± 2.3	23.4 ± 3.3
Winter active, T_a 19 °C	II	11	13.0 ± 1.4	15.9 ± 3.4
Winter active, T_a 4 °C (N3 to N7)	III	5	12.5 ± 1.3	11.5 ± 0.6
Winter active, T_a 4 °C (N1,N2,N8–N11)	III	6	< 4	3.3 ± 1.7
Winter torpid, T_a 4 °C	IV	7	< 4	4.0 ± 1.8

of methyl stearate. The nitroxide group of this probe locates in the hydrophobic region of the lipid bilayer [31] and the motion parameter can therefore be used as a comparative measure of membrane lipid fluidity. The motion of the label at 30 °C for each of the seasonal groups is shown in Table VII. Although there was a general increase in τ_0, indicative of a decrease in lipid fluidity, during winter and in torpor, the difference between the groups was not significant.

Discussion

In this study we have examined the effect of temperature on the molecular ordering of membrane lipids and the activity of succinate-cytochrome *c* reductase of liver mitochrondria of a mammalian hibernator. The data show two distinct changes in the thermal response of both the structural and functional properties of liver mitochondria, one seasonally-induced and the

TABLE VII

THE EFFECT OF SEASON AND TORPOR ON SPIN LABEL MOTION

Motion was measured using the spin label 9NSM with membrane lipids from squirrel liver mitochondria as described in the Methods and Materials section at 30 °C. *n* = number of animals in group.

Experimental group	Source of data (Table)	*n*	Mean T^*	Motion (τ_0)($\times 10^{10}$)
Summer field active (excluding J4,J5)	I	6	23	18.0 ± 1.3
Winter active, T_a 19 °C	II	12	13	19.3 ± 0.6
Winter active, T_a 4 °C (N3 to N7)	III	5	12	20.9 ± 0.9
Winter active, T_a 4 °C (N1,N2,N8–N11)	III	6	< 4	19.9 ± 0.6
Winter torpid, T_a 4 °C	IV	6	< 4	20.3 ± 1.25

other induced by low environmental temperature.

The first alteration in the thermal properties of these membranes consists of a lowering of the $T^{\star}$ in both structure and function by about 10 deg. C. In summer, active Richardson's ground squirrels exhibit a $T^{\star}$ of about 23°C (Table I), similar to that of many other mammalian species including mammalian hibernators in an active, normothermic phase [17,24,22]. Squirrels maintained in the laboratory during winter at a T_a of 19°C remained active but showed a mean $T^{\star}$ of 13°C (Table II). Thus this initial lowering of $T^{\star}$ and the concomitant increase in the E_a of succinate-cytochrome *c* reductase (Table V) occurred presumably without a change in body temperature, indicative of a seasonally-induced change in the thermal response of the membranes. The time at which this change occurs is variable, as shown by two individuals (J4 and J5, Table I) which were captured in summer but had a $T^{\star}$ of about 13°C. This is consistent with other field studies which have shown that the onset of hibernation in Richardson's ground squirrel varies greatly. Smith [32] reported that adult males were rarely seen after the end of June, adult females were ready for hibernation by mid-July, and juvenile males remained active until September or early October. Timing of the torpor cycle is related to sex and body weight [33,34] as well as to season.

The second alteration in membrane structure and function was detected in some animals maintained at T_a 4°C. In these individuals no transitions could be detected (Table III) and plots of spin label motion and membrane-associated enzyme activity were linear. It is assumed that the transition had shifted below the level of detection (about 4°C) because E_a for enzyme function in these animals was greater than the E_a above the transition in the remaining winter animals and in summer animals (Table V). Further evidence is obtained by analysing the data using the curvilinear equation of Klein [30]. As shown in Table VI, for those animals in which a transition could not be detected using linear analysis, this equation predicts a mean mid-point for the transition at 3°C.

Some of the animals which were exposed to 4°C and remained homeothermic had lowered their $T^{\star}$ to below 4°C (Table IV). In terms of the aspects of mitochondrial membrane structure and function examined in this study, these animals were indistinguishable from animals killed in torpor (Table IV). This supports the hypothesis that such changes are made in preparation for torpor and do not occur as a result of a direct, cellular response to the low body temperature of torpor. This implies a control mechanism, presumably endocrine, operating in two steps, or possibly two mechanisms, one shifting $T^{\star}$ from about 25°C to about 13°C and the other shifting $T^{\star}$ to below 4°C. It is likely that thyroid hormone is involved in this process, since it has been shown to affect mitochondrial membrane lipids [12] and to vary annually in such a way that circulating levels are low during the torpor season of hibernators [23].

There are insufficient data available on the transitions depicted by the change in E_a of membrane-associated enzymes and the spin label motion to speculate on changes in membrane lipid composition that might be responsible for the 10 deg. C shifts in the transition temperature. It is clear that these shifts in $T^{\star}$ occur as an 'all or nothing' process rather than a series of step-wise changes, since no value for a $T^{\star}$ was noted between 19°C, the lowest $T^{\star}$ in the summer field group, and 15°C, the highest $T^{\star}$ for the winter group. A similar gap of about 6 deg. C was evident between the lowest value for active winter animals, 10°C, (Table III), and the < 4°C for torpid winter animals (Table IV). Changes in lipid composition of liver mitochondrial membranes during torpor have been noted [35] but it was not possible to distinguish between the changes which might have been essential for torpor, and possibly related to the lowering of $T^{\star}$, and those which occurred as a result of the low body temperature experienced during torpor. Shifts in the $T^{\star}$ for enzyme function have been noted in ectotherms during acclimation to environmental temperatures [10]. For these animals, exposure to a lower temperature almost invariably results in an increase in the level of unsaturation of membrane lipids [36] and an increase in membrane lipid fluidity as measured by the spin labelling or fluorescence polarization [37].

Adjustments in the fluidity of membrane lipids have been proposed as a mechanism for maintaining the functional capacity of a cell at low temper-

ature. For ectotherms, the alteration in fluidity was found to compensate precisely for the temperature shift and the process was referred to as 'homeoviscous adaptation' [25]. There is no evidence that this type of process occurs in mammals even during the low body temperatures experienced in torpor, and no increase in fludity was observed with the mitochondrial membrane lipids of the Richardson's ground squirrels during torpor (Table VII). For the ground squirrel, torpor during winter is essential to conserve energy [38] and there would be little gained if membrane lipid fluidity increased to compensate for the reduction in rate of enzyme reactions at low temperature.

The significance of the lowering of $T^{\star}$ during hibernation is not clear. Induced hypothermia in mammals leads to a loss of potassium and other ionic gradients [15,39] which in myocardial tissue leads to ventricular fibrillation [41,42] at temperatures a few degrees below $T^{\star}$. This suggests that the change in molecular ordering depicted by the transition in function and spin label motion leads to a loss of membrane integrity. Since survival during torpor necessitates maintenance of ion regulation and cellular compartmentation at low body temperatures [42] the $T^{\star}$ needs to be reduced and remain below the lowest T_b likely to be encountered during a given phase in the animals annual thermo-regulatory cycle.

We therefore conclude that the cyclic changes in membrane structure and function, essential for successful hibernation, are those that lower $T^{\star}$ rather than alter membrane fluidity. While it seems reasonable to suggest that cyclic physiological control mechanisms operate to ensure that during periods of change in body temperature the membranes of hibernating mammals will not pass through a transition in lipid ordering, it is not clear why hibernators raise the $T^{\star}$ to about 23°C during their summer active phase or why this $T^{\star}$ is characteristic of endotherms as opposed to ectotherms. Indeed, from the viewpoint of mitochondrial membrane structure and function it is not the torpid phase of mammalian hibernation that is difficult to explain and understand but rather the summer active phase.

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