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Differences in the thermotropic behaviour of mitochondrial membrane respiratory enzymes from homeothermic and heterothermic endotherms

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Summary. 1. The thermal response of liver and heart mitochondrial succinate oxidase and succinate cytochrome *c* reductase of eleven homeothermic and six heterothermic species was compared.

2. Arrhenius plots of succinate oxidase and succinate cytochrome *c* reductase of homeotherms exhibited a mean T^* (Arrhenius critical temperature) of 22.6 °C. In normothermic heterotherms T^* occurred at a mean temperature of 20.1 °C. During the season in which they entered torpor, two of the heterothermic mammals exhibited a mean T^* at 10.9 °C, whilst for three species no T^* was observed in the temperature range 5 °C to 40 °C.

3. The lowered T^* in the heterothermic species was accompanied by an increase in the E_a and Q_{10} both above and below the discontinuity in the Arrhenius plot. The increase Q_{10} of the mitochondrial enzymes isolated from animals during torpor was identical to the Q_{10} for O_2 consumption for animals entering torpor. This may represent an important factor for energy conservation during torpor.

Introduction

The thermal behaviour of liver mitochondrial succinate oxidase is correlated with the thermoregulatory strategy in many mammals. Homeotherms and normothermic (active) heterotherms have been shown to exhibit discontinuities in Arrhenius plots (T^*), i.e. changes in activation energy (E_a) of succinate oxidase at temperatures between 20 °C and 28 °C (Lyons and Raison 1970; Raison and Lyons 1971; Roberts et al. 1972; McMurchie et al. 1973; Raison and McMurchie 1974; McMurchie and Raison 1979; Pehowich and Wang 1981; Geiser et al. 1984b). In contrast, for torpid heterotherms such as echidnas, the temperature at which T^* occurred, was lowered to between 6 °C to 10 °C (McMurchie and Raison 1975). Furthermore, in hibernating ground squirrels and torpid bats, no discontinuity was observed in the temperature range between 5 °C and 40 °C (Raison and Lyons 1971; Pehowich and Wang 1981; Geiser et al. 1984b), suggesting that T^* was lowered to a temperature below the assayed temperature range. A similar thermal response was observed with liver mitochondrial succinate cytochrome *c* reductase, a partial reaction of the mitochondrial electron transport chain (McMurchie et al. 1983a, b; Geiser et al. 1984a, b).

The lowered T^* of the mitochondrial respiratory enzymes provides a possible biochemical explanation for the ability of heterothermic mammals, in contrast to homeotherms, to withstand prolonged body temperatures (T_b) well below 20 °C and arouse spontaneously using endogenous heat production. The simultaneous increase in E_a could be considered as an important factor for energy conservation during torpor.

Since there is considerable disagreement regarding the interpretation of the above type of experiments which utilize data derived from Arrhenius plots (Charnock 1978; Willis et al. 1981), a comprehensive comparison of several homeothermic and heterothermic endotherms was conducted in this paper. The thermal response of liver and heart mitochondrial succinate cytochrome *c* reductase of two placental and five marsupial species was compared with data already existing in the literature. In addition, the thermal response

Abbreviations: E_a Arrhenius activation energy; T_b Body temperature; T_a Ambient temperature; T^* Arrhenius critical temperature

of liver mitochondrial succinate oxidase from a marsupial and a bird was also compared with values obtained from the literature.

Materials and methods

Homeotherms. Rats, hooded Wistar (*Rattus norvegicus*, Muridae, 200 g to 300 g) were maintained at an ambient temperature (T_a) of 23 °C with a commercial diet and water *ad libitum*. Guinea pigs (*Cavia aperea*, Caviidae, 500 g to 750 g) were supplied by the Institute of Medical and Veterinary Science, Adelaide, South Australia. Commercial diet and water were available *ad libitum*. Tammar Wallabies (*Macropus eugenii*, Macropodidae, 4 kg to 6 kg) were maintained in grassy animal yards at Macquarie University, Sydney, and fed with a supplementary pellet diet and water *ad libitum*. Tammars are competent homeotherms (Dawson et al. 1969). Euros (*Macropus robustus*, Macropodidae, approximately 30 kg) were kept under the same conditions as the Tammars and are homeothermic. Greater Gliders (*Petauroides volans*, Petauridae, 1 kg to 1.6 kg) were collected in Victoria during autumn and early winter (May/June). Animals were sacrificed and the livers were transported to the laboratory in liquid N_2. Greater Gliders are considered to be homeothermic (Rübsamen and Hume 1982). Brushtail Possums (*Trichosurus vulpecula*, Phalangeridae, approximately 2 kg) were caught in the Sydney area. Brushtail Possums are homeothermic (Dawson 1969). Domestic Fowl, White Leghorn (*Gallus gallus*, Phasianidae, approximately 2 kg) were purchased from a commercial hatchery. Commercial diet and water were available *ad libitum*. Mice (*Mus musculus*) from Geiser et al. (1984b) were included in the homeothermic group because they are homeothermic under normal laboratory conditions (Jacobson 1981).

Heterotherms. Brown Antechinus (*Antechinus stuartii*, Dasyuridae, 20 g to 55 g) were trapped in the Gosford area, New South Wales. They were maintained at 20 °C under a natural photoperiod. Minced meat, liver and water were available *ad libitum*. To induce torpor during winter (July/August), animals were placed in a room with a minimum T_a of 8 °C at night and maximum T_a of 20 °C during the day under natural photoperiod. When food was withheld for one night the animals usually entered torpor on the following morning. *A. stuartii* enters daily torpor only between April and September (autumn and winter in Australia) but not in summer (Wallis 1976).

Isolation and enzyme assays of mitochondria. The isolation of liver and heart mitochondria from individual animals and the enzyme assays were carried out as previously described (McMurchie et al. 1983a, b; Geiser et al. 1984b). A concentration of 10 mM succinate was used for all our assays, since the K_m for succinate cytochrome *c* reductase of mouse and *Antechinus* mitochondria determined above and below T^* for each species was similar and varied only slightly; ranges of concentrations were from 0.26 to 0.37 mM for all four determinations. This would indicate that true V_{max} rates were measured over the complete temperature range of the assays. Mitochondria were isolated from fresh tissue except for liver mitochondria from the Greater Glider which were isolated from frozen tissue. Freezing the tissue does not seem to affect the Arrhenius profile of succinate cytochrome *c* reductase from rat and mouse mitochondria (unpublished observations).

Arrhenius plots were constructed with 12 to 18 temperature points between about 5 °C and 38 °C. For succinate cytochrome *c* reductase, each of these temperature points was obtained using at least duplicate determinations (except for the *A. stuartii* heart mitochondria). Since most Arrhenius plots in the literature are fitted with straight lines, rather than curves, the method of least squares (Pollard 1977) was used for our data. For the determination of a change in slope the correlation coefficients and the residual sum of squares were calculated for all possible combinations of points fitted to two straight lines from the lower to the upper temperature extremes, using a computer program. A change in slope was considered to occur at the minimum for the sum of the residual sum of squares of the two straight lines. Tests for the significance of paired observations were made using the Student's *t*-test. All data shown in the text and tables are means ± standard error of the mean (SE).

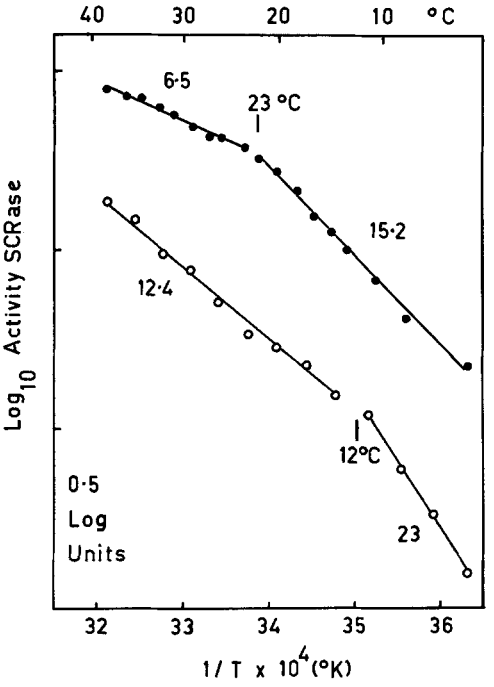

Fig. 1. Arrhenius plots of liver mitochondrial succinate cytochrome *c* reductase for the homeothermic Guinea Pig (•) and the heterothermic Brown Antechinus (o) isolated during winter. T^* is indicated in °C. The numbers adjacent to the lines are the Arrhenius activation energies (E_aI above T^* and E_aII below T^*) expressed in kcal/mol. The correlation coefficients for the line fits are: Guinea Pig, $E_a I = 0.985$, $E_a II = 0.994$; *Antechinus*, $E_a I = 0.989$, $E_a II = 0.998$. Each point represents the mean of at least two determinations. Specific activity at 37 °C for the Guinea Pig was 18 μmol/h·mg protein and 24 μmol/h·mg protein for the Brown Antechinus

Selection of data from existing literature. Measurements using tissue homogenates, rather than isolated mitochondria, and plots with less than 7 points were excluded. Furthermore, only those plots in which succinate was used as the respiratory substrate were included.

Results

Arrhenius plots of liver mitochondrial succinate cytochrome *c* reductase of a homeothermic mammal (Guinea Pig) and a heterothermic mammal

Table 1. Parameters associated with Arrhenius plots of mitochondrial succinate cytochrome *c* reductase from homeothermic mammals[a]

Species	T^*	E_aI	E_aII	Q_{10}[b]	Organ	Season	State	*n*	Reference
	(°C)	(kcal/mol)							
Rat (*Rattus norvegicus*)	23.0±0.3	4.2±0.6	15.5±0.6	1.25±0.20	L	S and W	Active	4	McMurchie et al. (1983a)
Rat (*Rattus norvegicus*)	22.7	6.1	14.1	1.38	H	S and W	Active	5	McMurchie et al.[c] (1983b)
Rat (*Rattus norvegicus*)	23.6±0.6	5.9±0.4	13.9±0.5	1.36±0.10	H	S and W	Active	7	This study
Mouse (*Mus musculus*)	22.6±0.7	6.8±0.6	16.9±1.4	1.44±0.13	L	S and W	Active	6	Geiser et al. (1984b)
Guinea Pig (*Cavia aperea*)	23.5±0.9	7.8±0.7	15.7±1.5	1.51±0.13	L	W	Active	3	This study
Brushtail Possum (*Trichosurus vulpecula*)	19.5	7.9	15.3	1.52	L	S and W	Active	2	This study
Greater Glider (*Petauroides volans*)	21.5	7.3	20.5	1.47	L	A and W	Active	2	This study
Tammar Wallaby (*Macropus eugenii*)	20.0±0	5.5±0.2	18.4±0.2	1.34±0.04	L	S and A	Active	3	This study
Euro (*Macropus robustus*)	23.0	5.4	19.0	1.33	L	S	Active	1	This study
Mean±SE	22.2±0.5	6.3±0.4	16.6±0.8	1.40±0.03 (9)					

[a] Data are expressed as the mean±SE where appropriate from the indicated number of observations (*n*) in either the summer (*S*), winter (*W*) or autumn (*A*) season of the year with mitochondria isolated from either liver (*L*) or heart (*H*). The number of separate determinations is shown in brackets

[b] Q_{10} between 30 °C and 40 °C

[c] Values have been obtained by normalizing five separate Arrhenius plots as described in that paper

(Brown Antechinus) isolated during the winter months, are shown in Fig. 1. The temperature at which a change in E_a is observed (T^*) for the Guinea Pig was about 23 °C which is significantly higher than the 12 °C evident for *A. stuartii*. The lower T^* for *Antechinus* was accompanied by an almost two-fold higher E_a value in the temperature region above T^* (E_aI) in comparison to the Guinea Pig. The value for the E_a below the T^* (E_aII) was also higher for *Antechinus*.

The close relationship between T^* and E_a for mitochondrial membrane associated respiratory enzymes and the thermoregulatory status of endotherms is also shown in Tables 1 to 4. Here are listed the thermal responses of liver and heart mitochondrial succinate cytochrome *c* reductase and succinate oxidase from eleven homeothermic and six heterothermic species (*Spermophilus* and *Citellus* are synonyms). The thermotropic behaviour of liver and heart mitochondria of homeotherms was not significantly different in any species, and are therefore treated as one group.

Homeotherms

The T^* of mitochondrial succinate cytochrome *c* reductase from the homeothermic species was 22.2±0.5 °C (mean±SE; $n=9$; range 19.5 °C to 23.6 °C). This value is similar to that observed for succinate oxidase from the homeotherms (22.8±1.0 °C; $n=9$; range 18 °C to 29 °C) (Tables 1 and 2). The mean E_a above the transition temperature T^* (E_aI) was 6.3±0.4 kcal/mol for succinate cytochrome *c* reductase and 5.9±1.2 kcal/mol for succinate oxidase, whilst the mean E_a below T^* (E_aII) was 16.6±0.8 and 18.2±1.6 kcal/mol, respectively. The value for the Q_{10} in the temperature range from 30 °C to 40 °C was 1.40±0.03 for succinate cytochrome *c* reductase (Table 1) and 1.41±0.10 for succinate oxidase (Table 2). The parameters associated with the thermal behaviour of liver mitochondrial succinate oxidase of the domestic fowl did not deviate from the values obtained for the mammals (Table 2). Since no significant difference in the value of the Q_{10}

Table 2. Parameters associated with Arrhenius plots of mitochondrial succinate oxidase from homeothermic mammals and a bird[a]

Species	T^* (°C)	E_aI (kcal/mol)	E_aII (kcal/mol)	Q_{10}[b]	Organ	Season	State	n	Reference
Rat (*Rattus norvegicus*)	18	9.2	24.4	1.70[c]	L	–	Active	1	Kemp et al. (1969)
Rat (*Rattus norvegicus*)	23.8	3.1	19.2	1.18	L	–	Active	2	Lyons and Raison (1970)
Rat (*Rattus norvegicus*)	22	2.9	19.5	1.17	H	–	Active	1	Lyons and Raison (1970)
Mouse (*Mus musculus*)	24.1±0.5	6.4±0.5	14.9±0.8	1.41±0.11	L	S and W	Active	6	Geiser et al. (1984b)
Rabbit (*Oryctolagus cuniculus*)	23	3.4	12.6	1.20	H	–	Active	1	McMurchie et al. (1973)
Sheep (*Ovis aries*)	29	2.0	10.0	1.10	L	–	Active	1	Raison and McMurchie (1974)
Squirrel monkey (*Saimiri sciurea*)	20	12.1	24.5	1.97[d]	L	–	Active	1	Roberts et al. (1972)
Tammar Wallaby (*Macropus eugenii*)	22.7	9.6	20.5	1.67	L	S and A	Active	2	This study
Domestic fowl (*Gallus gallus*)	22.4±0.5	4.3±0.7	18.5±0.7	1.26±0.20	L	W	Active	3	This study
Mean±SE	22.8±1.0	5.9±1.2	18.2±1.6	1.41±0.10 (9)					

[a] Data are expressed as the mean±SE where appropriate from the indicated number of observations (n) in either the summer (S), winter (W) or autumn (A) season of the year with mitochondria isolated from either liver (L) or heart (H). The number of separate determinations is shown in brackets

[b] Q_{10} between 30 °C and 40 °C. [c] Q_{10} above break. [d] Q_{10} between 20 °C and 34 °C

(or the other parameters) was observed between these two enzymes, they were treated as a single group when comparing the different homeothermic and heterothermic endotherms. When all species from Tables 1 and 2 were compared, a lower T^* for the homeothermic marsupials (21.3±0.7 °C) than for the placentals (23.5±1.2 °C) was observed. The mean values for E_aI (placentals 6.2±1.5 kcal/mol; marsupials 7.0±0.6 kcal/mol) and E_aII (placentals 16.1±2.0 kcal/mol; marsupials 18.6±1.1 kcal/mol) were not significantly different (data not shown). The difference between the T^* for succinate cytochrome c reductase of three placental (23.1±0.3 °C) and four marsupial (21.0±0.8 °C) species was more distinct ($p=0.06$).

Heterothermic mammals

The Arrhenius parameters for mitochondrial membrane-associated respiratory enzymes of the heterothermic mammals, including the hibernators, are shown in Tables 3 and 4. In their normothermic state or in the season in which they do not naturally enter torpor (usually summer), five of the six species investigated exhibited a similar thermal response for liver and heart mitochondrial succinate cytochrome c reductase and succinate oxidase as the homeothermic species (Tables 1 and 2). However, the mean T^* value was about 2 Celsius degrees lower in the heterothermic mammals (20.1±0.8 °C) when compared to the values for all homeotherms (22.6±0.7 °C) (Table 5). The response of the bent-wing bat differed from the other species in that it showed a constant E_a in summer. However, this species enters natural torpor in both winter or summer (Geiser et al. 1984b).

During torpor or post-arousal, two of the heterothermic mammals (*Antechinus* and the Echidna) exhibited a lowered T^* value with a mean value of 10.9 °C. This is considerably lower than the values obtained for both the homeothermic species and the normothermic heterotherms (Table 5). For all animals observed in torpor, a significantly higher E_aI (and Q_{10}) was observed in com-

Table 3. Parameters associated with Arrhenius plots of mitochondrial succinate cytochrome *c* reductase from heterothermic mammals[a]

Species	T^*	E_aI	E_aII	Q_{10}[b]	Organ	Season	State	*n*	Reference
	(°C)	(kcal/mol)							
Bent-wing Bat (*Miniopterus schreibersii*)	n.d.	14.9±1.1	–	2.20±0.16	L	S	Torpid	3	Geiser et al. (1984b)
Bent-wing Bat (*Miniopterus schreibersii*)	n.d.	11.4±0.9	–	1.80±0.14	L	W	Torpid	4	Geiser et al. (1984b)
Common Dunnart (*Sminthopsis murina*)	20.5	7.3	17.4	1.48	L	S	Active	2	Geiser et al. (1984a) (and unpublished)
Common Dunnart (*Sminthopsis murina*)	n.d.	16.9	–	2.50	L	W	Post-arousal	1	Geiser et al. (1984a)
Brown Antechinus (*Antechinus stuartii*)	21.4	5.5	20.4	1.34	L	S	Active	2	This study
Brown Antechinus (*Antechinus stuartii*)	12.9	11.4	22.7	1.83	L	W	Post-arousal	2	This study
Brown Antechinus (*Antechinus stuartii*)	14.4	13.9	32.9	2.10	H	W	Post-arousal	1	This study
Mean±SE (torpid or post arousal)	13.7 (2)	13.7±1.0 (5)	27.8 (2)	2.09±0.13 (5)					
Mean±SE (normothermic)	20.9	6.4	18.9	1.41 (2)					

[a] Data are expressed as the mean±SE where appropriate from the indicated number of observations (*n*) in either the summer (*S*) or winter (*W*) season of the year with mitochondria isolated from either liver (*L*) or heart (*H*). The number of separate determinations are shown in brackets.

[b] Q_{10} between 30 °C and 40 °C

n.d. not detected

parison to both homeotherms and normotherms (Tables 3, 4 and 5). When detected, E_aII was also higher. For most of the animals examined during torpor, no T^* was apparent and thus may have been below the assayed temperature range (Tables 3 and 4). For the hibernating hamster, a T^* at the same temperature as the active animals was observed. However, for these animals, E_aI was 1.5-fold higher when compared with active hamsters, and the increase in E_aII relative to E_aI was not as great as that observed for normothermic heterotherms (Tables 3 and 4). Furthermore, the change in E_a for the hibernating hamster was substantiated by only two temperature points below T^*.

The higher value of E_aI in the torpid heterothermic species (14.6±0.5 kcal/mol) was intermediate between E_aI (6.3±0.8 kcal/mol) and E_aII (17.2±1.1 kcal/mol) for the homeothermic species (Table 5), ($p<0.001$ and $p=0.08$, respectively; data not shown).

Discussion

The parameters associated with the thermal behaviour of liver and heart mitochondrial succinate cytochrome *c* reductase and succinate oxidase were significantly different between homeotherms and heterotherms, particularly for the latter during torpor. The variation of T^* between the various groups, i.e. homeotherms, normothermic heterotherms and torpid or post arousal heterotherms, as well as between placentals, marsupials and the monotreme, was generally correlated with the respective T_b range and set points for these various groups.

While homeothermic placentals have a precisely regulated T_b of about 38 °C (Herter 1956), homeothermic marsupials have a lower but also a constant T_b of about 36.5 °C (MacMillen and Nelson 1969; Dawson and Hulbert 1970). Thus while the mean T^* for all placentals was about 23 °C, the marsupials exhibited a slightly lower mean T^*

Table 4. Parameters associated with Arrhenius plots of mitochondrial succinate oxidase from heterothermic mammals[a]

Species	T^* (°C)	E_a I (kcal/mol)	E_a II	Q_{10}[b]	Organ	Season	State	n	Reference
Ground Squirrel (*Citellus lateralis*)	23	9.1	16.7	1.62	L	Sp	Active	1	Raison and Lyons (1971)
Ground Squirrel (*Citellus lateralis*)	n.d.	13.8	–	2.09	L	Sp	Torpid	1	Raison and Lyons (1971)
Ground Squirrel (*Spermophilus lateralis*)	19.7	10.1	18.8	1.72	L	–	Active	2	Pehowich and Wang (1981)
Ground Squirrel (*Spermophilus lateralis*)	n.d.	13.7	–	2.07	L	–	Torpid	2	Pehowich and Wang (1981)
Golden Hamster (*Mesocricetus auratus*)	20	9.9	20.5	1.76[c]	L	–	Active	1	Roberts et al. (1972)
Golden Hamster (*Mesocricetus auratus*)	20[d]	14.9	18.7	2.43[c]	L	–	Torpid	1	Roberts et al. (1972)
Bent-wing Bat (*Miniopterus schreibersii*)	n.d.	16.3±0.4	–	2.38±0.05	L	S	Torpid	3	Geiser et al. (1984b)
Bent-wing Bat (*Miniopterus schreibersii*)	n.d.	15.3±0.8	–	2.26±0.10	L	W	Torpid	4	Geiser et al. (1984b)
Common Dunnart (*Sminthopsis murina*)	n.d.	16.0	–	2.46	L	W	Post-arousal	1	Geiser et al., unpublished
Echidna (*Tachyglosssus aculeatus*)	17	8.0	18.0	1.60[e]	L	W	Active	1	McMurchie and Raison (1975)
Echidna (*Tachyglossus aculeatus*)	8.1	15.5	–	2.28	L	W	Torpid	2	McMurchie and Raison (1975)
Mean±SE (torpid or post-arousal)	8.1(1)	15.1±0.5(7)	18.7(1)	2.28±0.06(7)					
Mean±SE (normothermic)	19.9±1.2	9.3±0.5	18.5±0.8	1.68±0.04(4)					

[a] Data are expressed as the mean±SE where appropriate from the indicated number of observations (n) in either the summer (S), winter (W) or spring (Sp) season of the year with mitochondria isolated from liver (L). The number of separate determinations are shown in brackets

[b] Q_{10} between 30 °C and 40 °C

[c] Q_{10} measured in the range 20 °C to 34 °C

[d] 'Almost' a straight line Arrhenius plot, not included in calculation for mean values

[e] Q_{10} measured above T^*

n.d. not detected

of about 21 °C which was at the lower end of the range for all homeothermic species examined. For normothermic heterotherms with a T^* at 20 °C, a more variable T_b of about 36 °C has been reported (Herter 1956), whilst for the Echidna, a highly variable T_b of about 32.5 °C was observed (Augee 1978) and the T^* was at 17 °C. During daily torpor, the T_b of *A. stuartii* has been reported to vary between a minimum of 18 °C to 21 °C and a maximum of about 37 °C during activity (Wallis 1976). Deep hibernators survive T_b's as low as 0 °C to 5 °C during torpor and therefore have a T_b range of about 35 Celsius degrees (Herter 1956). During torpor or post-arousal, the T^*s of the two heterothermic mammals, *Antechinus* and the Echidna, were lowered to about 14 °C and 8 °C respectively, and the T^* of hibernating ground squirrels and the heterothermic *Spermophilus murina* and *Miniopterus schreibersii* was not detected in the temperature range between 5 °C and 40 °C. Thus the T^* of the group of heterotherms during torpor or postarousal was also positively correlated with the T_b during the hibernation/torpor cycle. A straight line fit to T_b versus T^* of the differ-

Table 5. Comparison of parameters of mitochondrial respiratory enzymes from homeothermic and heterothermic endotherms[a]

Group	T^*	E_aI	E_aII	Q_{10}[b]
	(°C)	(kcal/mol)		
1 Homeotherms	22.6±0.7 (11)	6.3±0.8 (11)	17.2±1.2 (11)	1.42±0.07 (11)
2 Heterotherms (normothermic)	20.1±0.8 (5)	8.1±0.8 (5)	18.8±0.7 (5)	1.56±0.07 (5)
3 Heterotherms (torpid or post-arousal)	10.9 (2)	14.6±0.5 (6)	23.3 (2)	2.23±0.08 (6)
1 vs 2	<0.05	NS	NS	NS
1 vs 3	n.d.	<0.001	n.d.	<0.001
2 vs 3	n.d.	<0.001	n.d.	<0.001

[a] Data are taken from Arrhenius plots of liver and heart mitochondrial succinate cytochrome *c* reductase and succinate oxidase activities as present in Tables 1–4. Data are presented as the mean±SE for the number of species indicated in brackets. Significance (*P*) has been determined by Student's *t*-test where appropriate with NS ($P>0.1$) being considered not significant

[b] Q_{10} between 30 °C and 40 °C

n.d. not determined

ent groups or species (excluding the species with a constant E_a) resulted in a correlation coefficient of 0.94. However, the findings of Roberts et al. (1972) suggest that during torpor of golden hamsters, the T^* is not lowered but the Arrhenius profile is altered to an almost straight line with an increased E_a value.

Where lowering of the T^* of the mitochondrial membrane respiratory enzymes occurred, it was accompanied by a significant increase in the value of both E_aI and Q_{10} in the temperature range above T^*. This is in agreement with the previously observed inverse relationship between T^* and E_a for mitochondrial membrane-associated respiratory enzymes in animals fed various lipid-supplemented diets (McMurchie and Raison 1979). Thus, irrespective of the conditions which induce changes in T^*, it would appear that the activation energy (particularly E_aI which occurs in the body temperature range) is closely linked with T^*. To date, emphasis has been placed on the importance of T^* in the survival of organisms at low temperatures, with the possibility that this critical temperature, which may arise as a result of some form of thermotropic phase transition in the mitochondrial membrane lipids (Raison and McMurchie 1974; McMurchie and Raison 1979), could limit survival of homeotherms during hypothermia (Lyons and Raison 1970; McMurchie et al. 1973). This view is supported by the correlation between the temperature-dependent survival and growth rates of hamster cells in culture and the associated thermotropic transition in the mitochondrial membrane lipids (Kruuv et al. 1983).

The effects of an elevation in the value of the activation energy and Q_{10} in the temperature range above T^*, associated with the altered T^*, should also be considered. In terms of the Q_{10}, an increase in this parameter would imply that the rate at which the mitochondrial membrane-associated respiratory enzymes decreased in activity with lowered temperature, would be increased. While for homeotherms a 10 Celsius degree lowering of T_b would result in only a 28% reduction in the activity of the membrane respiratory enzymes, a 65% reduction in activity could occur for torpid heterotherms when they experience the same drop in body temperature. During deep hibernation, a lowering of the T_b by about 35 Celsius degrees would be expected to lower the rate of the membrane-associated respiratory enzymes by about 95% in comparison to their rate at 37 °C (assuming substrate concentrations in excess of the K_m requirement). This possible magnitude of reduction approximates the decrease previously observed in the O_2 consumption of mammals during hibernation (Herter 1956; Swan 1974). The Q_{10} for O_2 consumption during entry into hibernation is between 2.1 and 2.3 (Swan 1974; Snapp and Heller 1981), which is similar in value to the mean Q_{10} obtained for the mitochondrial membrane-associated respiratory enzymes of torpid heterotherms (Table 5). Therefore the greater Q_{10} values observed for the heterotherms may be an important mechanism for energy conservation during mammalian torpor.

However there may be an advantage to the animal in having a low Q_{10} value for the mitochondrial membrane respiratory enzymes when normothermic. This may explain why the thermotropic properties of these enzymes of heterotherms at the end of the winter period, appear similar to those of homeotherms. At a constant T_b, a low Q_{10} value would ensure that if minor fluctuations in T_b were to occur, the reaction rates for mitochondrial oxidative phosphorylation, the process with which these respiratory enzymes are associated, would not be significantly affected.

The biochemical basis for the seasonal changes in the thermotropic behaviour of the mitochondrial membrane-associated respiratory enzymes may reside in changes in the physical properties of the membrane lipids induced by changes in the mem-

brane lipid composition. In support of this hypothesis it has been shown that the Arrhenius profile of such enzymes can be altered by changes in the composition of the membrane phospholipid fatty acids induced by dietary lipid treatments (McMurchie and Raison 1979; McMurchie et al. 1983a, b, c). Both increases and decreases in the saturation of phospholipids from various membranes of hibernators have been reported during hibernation (Aloia and Pengelley 1979). Furthermore, both an increase in membrane fluidity (McMurchie and Raison 1975; Charnock et al. 1980), a decrease (Keith et al. 1975; Cannon et al. 1975; Geiser et al. 1984b), as well as no change in fluidity (Cossins and Wilkinson 1982), has been measured for torpid or normothermic heterotherms in comparison to homeotherms.

Various thermotropic changes in the molecular ordering of membrane lipids have been detected by spin labelling and fluorescent probe techniques and the temperature at which these changes occur correspond closely with the T^* of the membrane-associated enzymes (Raison et al. 1971; McMurchie et al. 1973; Raison and McMurchie 1974; Brasitus et al. 1979; Livingstone and Schachter 1980; Whetton et al. 1982; Geiser et al. 1984a). Furthermore, the temperature at which these physical changes occur has been shown to be significantly lowered or even abolished during torpor (Keith et al. 1975; McMurchie and Raison 1975; Houslay and Palmer 1978; Augee et al. 1979; Raison et al. 1981; Geiser et al. 1984a, b). Thus there is considerable evidence that changes in the physical properties of membrane lipids could induce the changes observed in T^* as well as the activation energy E_a, as this latter parameter is also linked to the physical properties of the membrane lipids (McMurchie and Raison 1979). Besides compositional changes in the membrane lipids, alteration of the membrane physical properties could be achieved by a positional transfer of the acyl chains of the membrane phospholipids (Keough and Davis 1979).

A similar seasonal change in the thermotropic behaviour, i.e. mitochondrial membrane respiratory enzymes with a lower T^* and an increased Q_{10} during hibernation, has been reported for the soluble enzyme pyruvate kinase which involved changes in the isoenzyme pattern (Borgman and Moon 1976). Furthermore it has been hypothesized that the lowered intracellular pH during hibernation may be involved in the modulation of enzyme activity (Malan 1983), as demonstrated for phosphofructokinase of ground squirrels (Hand and Somero 1983). Since these findings do not exclude the possible lipid modulation of certain membrane-associated enzymes, it could be concluded that combined lipid and protein changes may be operative in longer term seasonal adaptation and form the basis for a short-term modulation by intracellular pH changes.

The results of this study support the notion that an increase in E_a and a lowering of T^* for the mitochondrial membrane-bound respiratory enzymes is associated with mammalian torpor. The results also suggest that despite the almost certain polyphyletic origin of torpor in monotremes, marsupials and placentals, a similar mechanism for adjusting mitochondrial membrane function has developed to meet the demands for energy conservation or survival during torpor.

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