

# Arrhenius parameters of mitochondrial membrane respiratory enzymes in relation to thermoregulation in endotherms

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**Summary.** 1. The relationship between the body temperature ( $T_b$ ), the Arrhenius critical temperature ( $T^*$ ), and the apparent activation energy above  $T^*$  ( $E_a1$ ), of liver and heart mitochondrial respiratory enzymes from eleven homeothermic and eight heterothermic species was determined using a linear regression analysis.

2. An inverse relation was observed between  $T^*$  and  $E_a1$  during torpor and hibernation.

3. In all thermoregulatory states,  $T^*$  decreased with  $T_b$  and  $T^*$  was equal to or below  $T_b$ .

4. During torpor  $E_a1$  increased in a linear manner as  $T_b$  was lowered.

5. It appears that the above Arrhenius parameters are closely linked to the thermoregulatory state of endotherms and thus may represent an adaptation for function at low  $T_b$ 's.

## Introduction

An alteration in the temperature-activity profile of mitochondrial membrane-associated respiratory enzymes has been observed during mammalian torpor and hibernation (Raison and Lyons 1971; Pehowich and Wang 1984; Geiser and McMurchie 1984). This change is characterized by a lowering or even disappearance of the Arrhenius critical temperature ( $T^*$ ) and a concomitant increase in the apparent activation energy ( $E_a$ ) of such enzymes, when compared with normothermic (summer-active) hibernating or heterothermic mammals or homeothermic species (Raison and Lyons 1971; McMurchie and Raison 1975; Pehowich and

Wang 1981, 1984; McMurchie et al. 1983a, b; Geiser et al. 1984b, c; Augee et al. 1984). The inverse relationship between  $T^*$  and  $E_a$ , associated with mammalian torpor, seems to hold for monotremes, marsupials and placentals, and is characterized by a lowering of  $T^*$  to a temperature apparently below that experienced by the animal during torpor. This alteration in the thermal response of mitochondrial respiratory enzymes during torpor may form an adaptation for function at low  $T_b$ , and provide a possible biochemical explanation as to why heterothermic, unlike homeothermic, species survive prolonged hypothermia, conserve energy whilst torpid and are able to arouse from torpor by means of endogenous heat production.

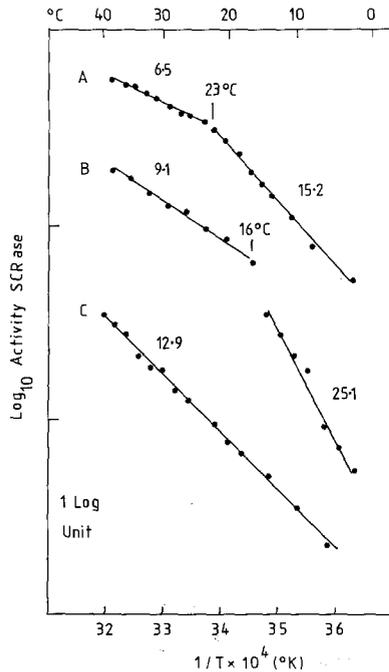
Since the low  $T^*$  and increased  $E_a$  appear to be somehow linked with low  $T_b$ , we have tested the possible significance of the relationship between (1)  $T^*$  versus  $E_a$ , (2)  $T^*$  versus  $T_b$  and (3)  $T_b$  versus  $E_a1$  (the apparent activation energy in the temperature region above  $T^*$ , which is within the  $T_b$  range) of eleven homeothermic and eight heterothermic species whilst they exhibited different thermoregulatory states.

## Materials and methods

The present paper compares the Arrhenius parameters of liver and heart mitochondrial succinate oxidase-system, succinate: cytochrome *c* reductase, succinate oxidase-linked proton ejection and  $Ca^{2+}$  uptake. Data are taken from: Kemp et al. (1969); Lyons and Raison (1970); Raison and Lyons (1971); Roberts et al. (1972); McMurchie et al. (1973); Raison and McMurchie (1974); McMurchie and Raison (1975); Pehowich and Wang (1981); McMurchie et al. (1983a, b); Geiser et al. (1984a, b), as summarized in Geiser and McMurchie (1984), who reviewed the above data with additional species, and from two comprehensive studies on ground squirrel mitochondria (Pehowich and Wang 1984; Augee et al. 1984). The graphs in the present paper were constructed from 65 separate experimental groups on 19 species which total over 250 individual Arrhenius plots. The species were: *Gallus gallus*, *Tachyglossus aculea-*

*Abbreviations:*  $E_a$  apparent activation energy;  $T^*$  Arrhenius critical temperature;  $T_b$  body temperature

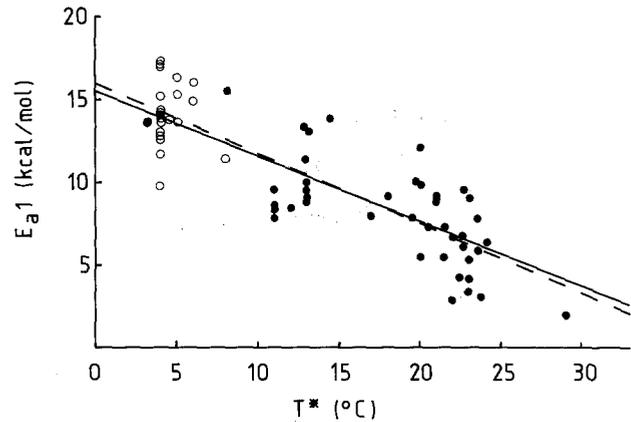
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**Fig. 1.** Arrhenius plots show the temperature dependence of liver mitochondrial succinate:cytochrome *c* reductase activity for the homeothermic guinea pig (*A*), the heterothermic brown antechinus (*B*) and the heterothermic bent wing bat (*C*), isolated during winter.  $T^*$  is indicated in  $^{\circ}\text{C}$ , the numbers adjacent to the lines are the apparent activation energy ( $E_{a1}$  above  $T^*$  and  $E_{a2}$  below  $T^*$ ) expressed in kcal/mol. The lines were fitted to the data by least squares regressions and the coefficients of determination ( $r^2$ ) for each linear region above the below  $T^*$  were between 0.97 and 0.99. Data were taken from: *A* Geiser and McMurchie (1984); *B* unpublished; and *C* Geiser et al. (1984b)

*tus*, *Antechinus stuartii*, *Sminthopsis murina*, *Petauroides volans*, *Trichosurus vulpecula*, *Macropus eugenii*, *Macropus robustus*, *Miniopterus schreibersii*, *Saimiri sciureus*, *Rattus norvegicus*, *Mus musculus*, *Cavia aperea*, *Citellus lateralis*, *Spermophilus columbianus*, *Spermophilus richardsonii*, *Mesocricetus auratus*, *Oryctolagus cuniculus*, and *Ovis aries*.

There is considerable controversy as to whether data on the variation of the logarithm of enzyme activity as a function of the reciprocal of the absolute temperature should be considered as one or two linear-related functions or as a continuously variable function. In most of the cases cited above, straight lines rather than curves were fitted to the Arrhenius plots to determine the temperature for the change in  $E_a$  (i.e.  $T^*$ ). In several instances, curves were fitted using the equations of Silvious and McElhanev (1980) or Klein (1982), but no significant differences between  $T^*$  and  $T_0$  (the temperature at which the enzyme is equally distributed between its high and low activity forms) or  $T^*$  and  $T_m$  (the midpoint of the transition) were observed (McMurchie et al. 1983b; Geiser et al. 1984b, c; Augée et al. 1984). Regression analyses were performed which either excluded or included Arrhenius plots with a constant  $E_a$  over the assayed temperature range "straight line plots". For the regression analysis which included plots with a constant  $E_a$ , the temperature of the lowest point determined was assumed to be the  $T^*$ . In Augée et al. (1984) straight line plots were fitted by the equation of Klein (1982), which resulted in  $T_m$  values within 1 centigrade degree of the lowest measured point. These points were included in the  $T^*$  values in Figs. 2



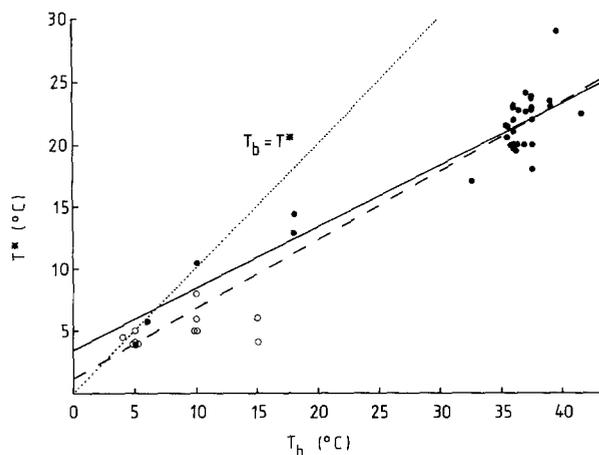
**Fig. 2.** The relationship between  $E_{a1}$  and  $T^*$  of mitochondrial respiratory enzymes. The lines represent fits to a) the  $T^*$ 's (●), determined from non-linear Arrhenius plots (solid line), and b) including the lowest temperature point (○) of linear plots (dashed line) as described in Materials and methods. The equations are: (a)  $y = 15.43 - 0.395x$  (correlation coefficient 0.73), (b)  $y = 15.96 - 0.421x$  (correlation coefficient 0.84)

and 3. For the plot of liver mitochondrial succinate oxidase from hibernating golden hamsters (Roberts et al. 1972), a constant  $E_a$  was assumed because an almost linear plot was observed. Where  $E_{a1}$  or  $T^*$  was compared with  $T_b$  only the summer field and hibernating ground squirrels from Pehowich and Wang (1984) and Augée et al. (1984) were used because it is not known if the animals in the other groups, which represent transition states between normothermia and hibernation, entered shallow torpor. Body temperatures for the different species in their particular physiological states were taken from the original papers where indicated. For those species in which no  $T_b$  was measured, other sources were used to obtain  $T_b$ . Thus the  $T_b$ 's for the homeothermic placentals and the domestic fowl were taken from Altman and Dittmer (1973); those for the homeothermic marsupials from Dawson and Hulbert (1970) where present; *Miniopterus schreibersii* in torpor (Kulzer et al. 1970); *Petauroides volans* (Rübsamen et al. 1984); *Sminthopsis murina* (Geiser et al. 1984c); *Antechinus stuartii* (Wallis 1976); *Tachyglossus aculeatus* normothermic (Augée 1978).

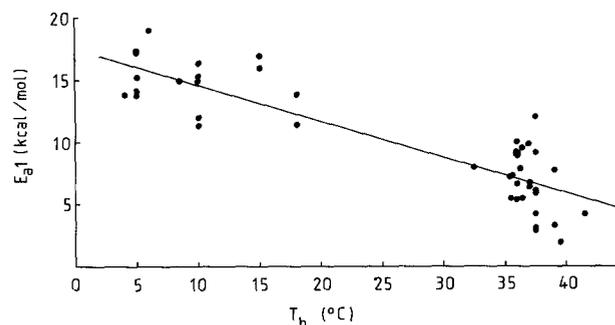
The relationship between  $T^*$  versus  $E_{a1}$  and  $E_{a2}$ ,  $T^*$  versus  $T_b$  and  $E_{a1}$  versus  $T_b$  for the different species in corresponding physiological states was tested by linear regression analysis, using the correlation coefficient calculated for the line fit. Differences in slope and elevation of the different lines were also determined.

## Results

Representative Arrhenius plots of liver mitochondrial succinate:cytochrome *c* reductase from a homeothermic mammal (guinea pig) and two heterothermic mammals, the brown antechinus *Antechinus stuartii*, an insectivorous marsupial, and the bent-wing bat, *Miniopterus schreibersii*, in winter, are shown in Fig. 1. The temperature at which a change in  $E_a$  was observed ( $T^*$ ) occurred at  $23^{\circ}\text{C}$  for the homeothermic guinea pig and at about  $16^{\circ}\text{C}$  for the heterothermic antechinus, which enters shallow daily torpor in winter (Wallis 1976).



**Fig. 3.** The relationship between  $T_b$  and  $T^*$ . The lines represent fits to a) the  $T^*$  (●) determined from non-linear Arrhenius plots (solid line) and b) including the lowest temperature point (○) of linear plots (dashed line) as described in Materials and Methods. The dotted line represents  $T_b = T^*$ . The equations are: (a)  $y = 3.43 + 0.50x$  (correlation coefficient 0.93), (b)  $y = 1.20 + 0.56x$  (correlation coefficient 0.97)



**Fig. 4.** The relationship between  $T_b$  and  $E_{a1}$ . The equation is:  $y = 17.53 - 0.29x$  (correlation coefficient 0.87)

A constant  $E_a$  was observed for the bat *Miniopterus schreibersii*, which hibernates in Australia (Hall 1982), and it was assumed that  $T^*$  occurs at or below the lowest temperature assayed. The lower  $T^*$  (or constant  $E_a$ ) for the antechinus and the bat, when compared with the guinea pig, was accompanied by about 1.5- and 2-fold higher  $E_a$  values, respectively, in the temperature region above  $T^*$  ( $E_{a1}$ ).

The relationship between  $T^*$  and  $E_{a1}$  was tested for multiple investigations on 19 endothermic species (Fig. 2). The points represent the separate experimental groups as described in Materials and Methods. A linear regression analysis was performed both on plots where a  $T^*$  was detected and thus excluded those plots with a constant  $E_a$ , as well as on all plots where it was assumed that  $T^*$  occurred at the lowest assay point for each species from those plots which were represented by

straight lines. As shown in Fig. 2, both fits resulted in a similar equation. The correlation coefficient was greater when all forms of Arrhenius plots were included, with no significant difference in either the slope or the elevation between the two lines. The determination of  $T^*$  from linear plots (calculated from those plots where a  $T^*$  was present) was:

$$T^* = (15.43 - E_{a1}) / 0.395 \quad (1)$$

where  $T^*$  is the temperature at which a change in  $E_a$  occurs and  $E_{a1}$  is the Arrhenius activation energy in the temperature region above  $T^*$  in kcal/mol. If Eq. (1) is applied to the data of Pehowich and Wang (1984) for hibernating ground squirrels, in which a mean constant  $E_a$  of 16.6 kcal/mol is observed (linear plot), a  $T^*$  of  $-3.0^\circ\text{C}$  would be obtained. The correlation between  $T^*$  and  $E_{a2}$  (the Arrhenius activation energy in temperature region below  $T^*$ ) was also investigated.  $E_{a2}$  increased with a lowering of  $T^*$  ( $E_{a2} = 23.51 - 0.296 T^*$ ; data not shown) but the correlation coefficient for this linear fit was 0.35. Thus it seems that  $T^*$  and  $E_{a2}$  are not closely related. However, one has to consider that  $E_{a2}$  is frequently based on only a few temperature points, especially when  $T^*$  is low. At steeper slopes,  $E_a$  approaches infinity and therefore slight variations in the activity measurements and the subsequent line fits would result in large errors.

The lowering of  $T^*$  appears to be related to the body temperatures of the different mammalian species during changes in their thermoregulatory state (Geiser and McMurchie 1984). In Fig. 3 this relationship was tested using linear regression analysis. Regression lines were fitted which both included and excluded the lowest point for linear Arrhenius plots. In both instances similar equations were obtained which differed only in their y-intercept values. Slope and elevation of the two lines were not statistically different. The constructed line  $T_b = T^*$  shows that all points are equal to or lower than  $T_b$  with the exception of one  $T^*$  and one lowest temperature point, which are within  $0.5^\circ\text{C}$  of  $T_b$ . The difference between  $T_b$  and  $T^*$  was more apparent during both the normothermic period of heterothermic species and for the homeothermic species exhibiting a  $T^*$  at about  $20^\circ\text{C}$ .

Since  $T^*$  and  $E_{a1}$  are closely related (Fig. 2), the correlation of  $T_b$  and  $E_{a1}$ , which is within the body temperature range, was also tested. As shown in Fig. 4, the groups were distributed in two regions of the graph. High  $T_b$ 's were associated with low  $E_{a1}$  values and low  $T_b$ 's with high  $E_{a1}$  values. However, a linear fit appears to be appropriate considering the value of the correlation coefficient.

## Discussion

The results of this comparative analysis show the close relationship between the Arrhenius critical temperature ( $T^*$ ) and the apparent activation energy ( $E_a$ ) of liver and heart mitochondrial membrane-associated respiratory enzymes. Both Arrhenius parameters correlate with the body temperatures of the different species when they experience particular physiological states.  $T^*$  and  $T_b$  show an interesting relationship in that all but two of the body temperatures observed are equal to or greater than  $T^*$ . These two points (one  $T^*$  determined from a non-linear plot, and one lowest temperature point of a linear plot), which are lower than  $T_b$ , are within 0.5 centigrade degrees of  $T_b$ . Considering that such activity measurements are usually taken in 2 to 3 centigrade degree intervals,  $T^*$  could easily be 1 to 2 centigrade degrees lower than indicated and therefore subsequently occur at a temperature lower than the reported  $T_b$ . The gaps in  $T^*$  and  $E_a$  at  $T_b$  of 20 to 30 °C observed in Figs. 3 and 4 are due to no corresponding information about species which enter shallow torpor in this temperature range.

It has not been determined if mitochondria from tissues other than liver and heart show similar relationships. A comparative study of kidney mitochondrial succinate oxidase from homeothermic mammals (dogs, pigs and humans) indicates that the  $T^*$  occurs between 15 and 20 °C (Southard et al. 1983), similar to liver and heart mitochondrial respiratory enzymes from a variety of other homeotherms. In contrast, rabbit kidney mitochondrial succinate oxidase exhibited a  $T^*$  between 10 and 12° C (Southard et al. 1983). Despite this observation, the inverse relationship between  $E_a$  and  $T^*$  also appears to occur in kidney mitochondria. Unfortunately, the temperature intervals in the Arrhenius plots of Southard et al. (1983) were 5 centigrade degrees and it is therefore not possible to determine the temperature for  $T^*$  with precision. Furthermore, the apparent activation energies ( $E_a$ ) of kidney mitochondrial succinate oxidase (Southard et al. 1983) were between 2.2- and 3.3-fold higher than observed for liver and heart mitochondria from homeotherms (Geiser and McMurchie 1984), and no value for the respiratory control ratio of isolated kidney mitochondria was given.

The molecular basis for the change in the apparent activation energy of these membrane-associated enzymes remains controversial. Several studies on isolated mitochondrial membranes or the extracted lipids using spin labelling techniques have revealed phase transition temperatures coinci-

dent with the change in  $E_a$  determined from enzymic studies (Keith et al. 1975; McMurchie and Raison 1975, 1979; Geiser et al. 1984b, c; Augee et al. 1984). These observations support the original hypothesis of Raison et al. (1971) that the increase in  $E_a$  has as its basis some temperature-induced change in the molecular ordering of the membrane lipid components. Since the non membrane-associated enzyme pyruvate kinase also show a similar thermal response with lowering of  $T^*$  during hibernation (Borgman and Moon 1976), it has been suggested by others that changes in the  $E_a$  of some membrane-associated enzymes may be due to a conformational change in the enzyme proteins. Furthermore, the existence of isoenzymes with different temperature optima and responses could explain the increase in  $E_a$  at low temperatures in certain circumstances. However, it has been shown that the thermal response of the mitochondrial membrane-associated respiratory and energy-transducing enzymes can be altered by the nature of the dietary lipid intake, which markedly affects the lipid composition of the mitochondrial membrane (McMurchie and Raison 1979; Innis and Clandinin 1981; McMurchie et al. 1983a, b, c; Robblee and Clandinin 1984). Other influences like substrate inhibition as observed for *Acholeplasma*  $\text{Na}^+$   $\text{Mg}^{2+}$ -ATPase (Silvius and McElhaney 1980) are unlikely for these enzyme systems (Geiser et al. 1984b; Geiser and McMurchie 1984).

The fact that  $T^*$  is lowered during the preparation for hibernation (Pehowich and Wang 1984; Augee et al. 1984) would suggest that this represents an adaptive rather than a consequential response to hibernation. The physiological advantage of a low  $T^*$  and a high  $E_a$  during torpor, may reside in the ability of these animals to function over a much greater temperature range than is usually observed for homeothermic species, and also to conserve energy whilst torpid. The almost 2-fold greater  $E_a$  (or  $Q_{10}$ ) values in torpid animals, in comparison to homeotherms and normothermic heterotherms, would result in a significantly greater reduction in the rate of oxidative phosphorylation as the body temperature is lowered. This would explain the increased temperature-induced reduction in the absolute mitochondrial respiratory rates observed in torpid hibernators when compared with values obtained during their normothermic phase (Chaffee 1962; Roberts et al. 1972; Pehowich and Wang 1984). Furthermore, the increased  $Q_{10}$  would favour thermogenesis during arousal (Roberts et al. 1972).

The results of this study support the notion that the  $T^*$  and  $E_a$  of mitochondrial membrane

respiratory enzymes are closely linked. The body temperatures of endotherms, whether active or torpid, appear to be regulated above  $T^*$ . The low  $T^*$  during torpor may represent an adaptation for function or arousal at low  $T_b$  and the accompanying change in  $E_a$ , when considered in terms of  $Q_{10}$ , may have relevance to energy conservation during torpor.

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