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# Temperature Activation of Rate Limiting Steps of Mitochondrial Respiration

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*Abstract*

Studies of the temperature dependence of the energy of activation of rat liver mitochondrial respiration have revealed a linearity in the energy of activation between 25 to 41° with changes in slope observed at 20–25° and higher than 41°. Analysis of the phase transition and energy of activation of coupled and uncoupled respiration affords an approach to clarification of mechanisms.

## *Introduction*

There is evidence that the temperature dependencies of mitochondrial respiration are species specific and are correlated with the environmental temperature conditions of organisms [1–3] or with their physiological states.[4, 5] Previous studies of thermal effects on mitochondria have emphasized the stability rather than kinetics of processes.[6–9] Analysis of Arrhenius plots may reveal a general sequence of rate limiting steps of mitochondrial metabolism. This investigation of the temperature range and activation energy of rat liver mitochondrial respiration indicates the existence of definite rate limiting steps in the process.

## *Materials and Methods*

Male rats were reared on standard diets at 20° under a 12:12 h light–dark rhythm. To prevent fluctuations stemming from circadian rhythms [10] animals were always sacrificed 2 h after the onset of light. Liver mitochondria were isolated in a 300 mM sucrose medium containing 5 mM Tris-Cl at pH 7·5 as previously described.[11] Respiration was measured in a reaction mixture containing 120 mM sucrose, 4 mM Tris-Cl, 5 mM $MgCl_2$, 8 mM KCL, 2mM Na phosphate, 5 $\mu$g/ml rotenone, 3 mM Na succinate and mitochondria (0·8 mg protein/ml) at pH 7·4.

Oxygen consumption was measured polarographically; data are given as $\mu$M $O_2$ utilized/mg protein/min. A closed vessel was employed to prevent back diffusion of $O_2$ into the reaction mixture. Temperature was controlled during experiments by circulating water through a jacket surrounding the cuvette. The actual temperature of the reaction mixture was measured by means of a thermosistor probe. $O_2$-Partial pressure of the reaction mixture was allowed to equilibrate at the reaction temperature prior to

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commencing experiments. The response of the $O_2$ electrode was calibrated at each temperature employed. Linear rates of $O_2$ consumption were recorded at all temperatures measured. Non-linear rates of $O_2$ consumption, presumably resulting from denaturation were observed only at higher temperatures and after long incubation times (viz. after 10 min at 41°).

*Results and Discussion*

The Arrhenius plots calculated for $O_2$ consumption in coupled and uncoupled states are shown in Fig. 1. Coupled respiration was assayed in the absence and presence of ADP. Uncoupled mitochondria were either treated with 30 $\mu$M DNP or an aged mitochondrial preparation was employed. The aged mitochondria were prepared by keeping a preparation for 24 h after isolation at 0° in a stock suspension containing 53 mg protein/ml. Respiratory control was absent in the aged preparation, but the fresh and coupled preparations showed good respiratory control which was dependent upon temperature (cf. Fig. 2).

The results (Fig. 1) show a relatively wide temperature range over which the Arrhenius plot is linear, suggesting a single rate limiting step. The temperature range over which

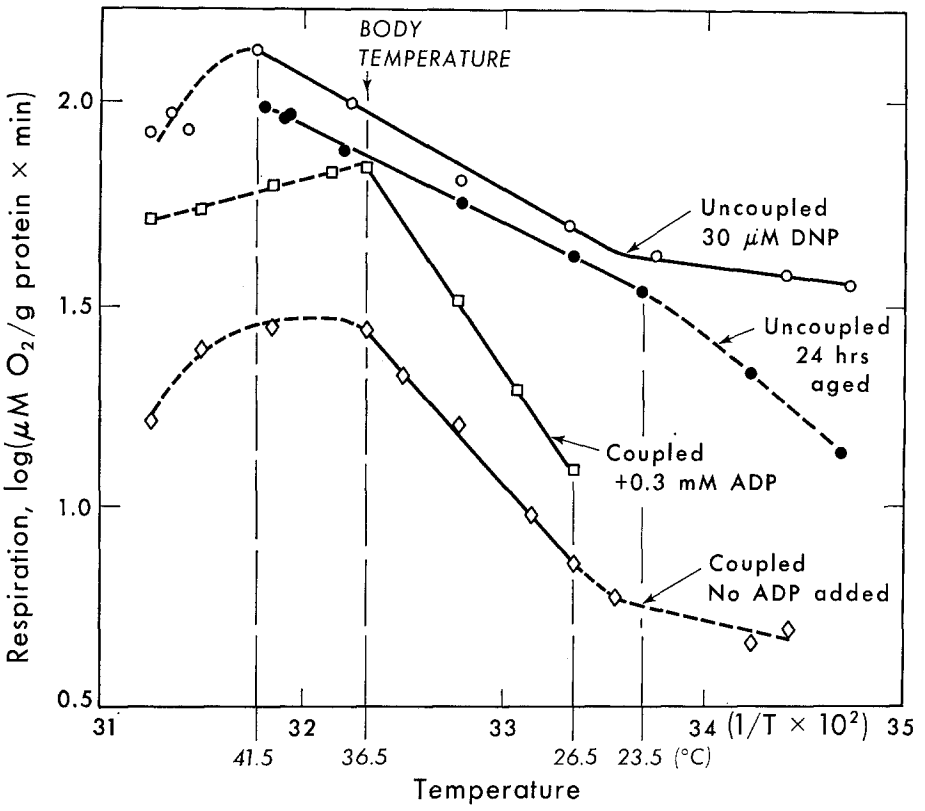


Figure 1. Arrhenius plot of succinate respiration by rat liver mitochondria. The reaction mixture contained 120 mM sucrose, 4 mM Tris-Cl, 8 mM KCl, 4 mM $MgCl_2$, 2 mM Na phosphate, 1·5 $\mu$g rotenone/ml, 0·8 mg protein/ml mitochondria at pH 7·4. Other conditions as described in the text.

the Arrhenius plot is linear is within a shorter range (26·5–36·5°) for coupled respiration than for the uncoupled respiration (23·5–41°). Of further interest is the finding of a break in this plot for coupled respiration at body temperature. This break in the curve is not the result of inhibition of electron flow *per se* because the linearity of uncoupled respiration is unchanged up to 41°. Only at temperatures higher than about 41° does the decay rate become superimposed over the residual respiration. Figure 2 show that respiratory control increases slightly in the range just above body temperature before it finally declines above 41°.

Break points in the Arrhenius plots at lower temperatures are also observed. It seems possible that the transition at 26·5 and 23·5° indicates a temperature range where

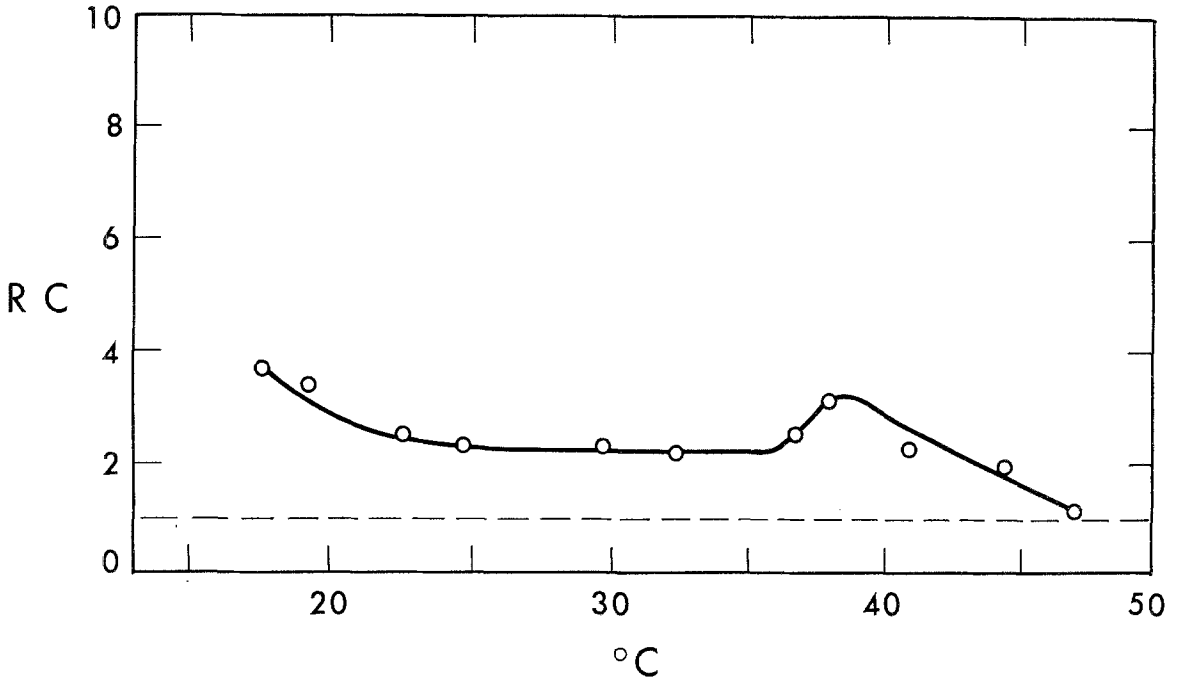


Figure 2. Temperature dependence of mitochondrial respiratory control (RC). The RC is taken from the respiratory rates after the second addition of a small amount of ADP to the same sample. Other conditions as in Fig. 1.

structural components of the membrane are changed. Other experiments have shown a similar transition point at about 24° during ion transport under oscillatory state conditions and in the passive osmotic response of mitochondria. Lyons and Raison[3] report a transition in the Arrhenius plot of respiration at 10° for plant mitochondria isolated from species sensitive to chilling injury.

From the foregoing it seems evident that the use of "room temperature" ranging between 20–25° is an unreliable range in which to perform experiments with rat liver mitochondria because slight variations in temperature may cause a large shift in the kinetics of respiration. For example, confusion could arise if results of experiments made at 20° were compared to those at 25°. It seems reasonable to suggest 30° as an optimum temperature where the rate limiting steps appear stable under all conditions. At 30° the decay rate is negligible if the experiments are performed within 10 min.

TABLE I. Activation energy of rate limiting steps of succinate respiration in rat liver mitochondria (Data are calculated from Fig. 1)

| State of activity | Test condition | Arrhenius plot linear temperature range (°C) | Activation energy (cal/mole) |
|---|---|---|---|
| Coupled, phosphorylating | ADP present | 26·5–36·5 | 33,500 |
| Coupled, non-phosphorylating | ADP absent | 26·5–36·5 | 25,400 |
| Uncoupled | DNP present | 23·5–41·5 | 13,000 |
| | | 15·0–23·5 | 3,250 |
| Uncoupled | Aged preparation | 23·5–41·5 | 10,800 |

The activation energies are summarized in Table I. Under uncoupled conditions the activation energy lies within a range normal for chemical kinetics (3–13,000 cal/mole). An unusually high activation energy for coupled respiration, especially under phosphorylating conditions, suggests the existence of some kind of cooperative effect. The rate limiting step seen in coupled respiration may not be connected with the phosphorylation process itself. Hall and Arnon[12] have shown that cyclic photophosphorylation in chloroplasts is temperature-independent within the range of −10 to +15°. These results may arise because the phosphorylation reaction is rapid enough over that temperatrue range, and the electron transport reactions limit ATP synthesis. These observations suggest that the phosphorylation step may have a very low activation energy. Examination of the rate limiting steps at individual sites of phosphorylation along the respiratory chain may answer the question whether the high activation energy of succinate respiration in mitochondria is caused by coupling to phosphorylation steps or to other accessory processes. Further experiments are proposed to determine the energy of activation of energy-linked processes arising from electron flow through restricted spans of the respiratory chain.

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