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CHANGES IN RESPIRATORY CONTROL AND CYTOCHROMES IN LIVER MITOCHONDRIA DURING HIBERNATION

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SUMMARY

Liver mitochondria from the ground squirrel, *Citellus lateralis*, were compared during states of hibernation and non-hibernation. During hibernation a decreased total amount of mitochondrial cytochrome was noted. In addition changes in the relative amounts of cytochromes *b* and *c* and a pronounced decrease in cytochrome a–a_3 was shown by difference spectra. The rate of oxygen uptake with succinate and ADP was less in the hibernator. However, addition of the uncoupler, salicylanilide XIII, stimulated oxygen consumption of the hibernator to a rate greater than that observed in the non-hibernating animal indicating that oxygen uptake was not limited by the cytochrome concentration. It is postulated that the sluggish rate of oxygen uptake under phosphorylating conditions by liver mitochondria of the hibernator may be caused by a change in the penetration of ADP and/or P_i, or an alteration in some parameter of the mechanism of coupled phosphorylation.

The hibernating ground squirrel has been utilized as part of a comparative biochemical study relating certain metabolic changes to decreased oxygen consumption. A marked decrease in body temperature and metabolic rate characterize mammalian hibernation. However, while changes in mitochondrial oxidative capacity are consistently observed in the hibernator, there is apparently normal phosphorylating efficiency[1]. In addition prolonged fasting contributes to active gluconeogenesis in the liver and kidney of the hibernating animal[2]. The present communication compares some properties of liver mitochondria isolated from the hibernating and active golden mantled ground squirrel, *Citellus lateralis*. Marked differences were observed in the cytochrome spectra and content as well as respiratory control.

Hibernating animals weighing between 250–280 g were housed individually in complete darkness at 6° with access only to water. Hibernation occurred in November and was allowed to continue for approx. 3 months before the animals were sacrificed. Active animals (non-hibernating) were used as controls. Mitochondria prepared by the procedure of SCHNEIDER[3] were suspended in 0.25 M sucrose at a concentration equivalent to 1 g liver per ml. Difference spectra were obtained with an Aminco Chance dual-wavelength spectrophotometer. A collodian-coated, Aminco platinum electrode designed to fit the spectrophotometer was used to measure oxygen concen-

tration in a 3.0-ml cuvette. The reaction was run at 22° and pH 7.4. Hemoproteins were determined quantitatively as pyridine hemochromogens[4]. Protein was measured by the biuret method[5] after solubilization of the mitochondria with deoxycholate, 0.13 % weight/vol.

Mitochondrial cytochrome components of a number of hibernating and active animals were examined with a dual-wavelength spectrophotometer. The spectra shown in Fig. 1 are typical of the results obtained and indicate that the mitochondrial cytochromes of non-hibernating ground squirrel liver are similar to those of the rat.

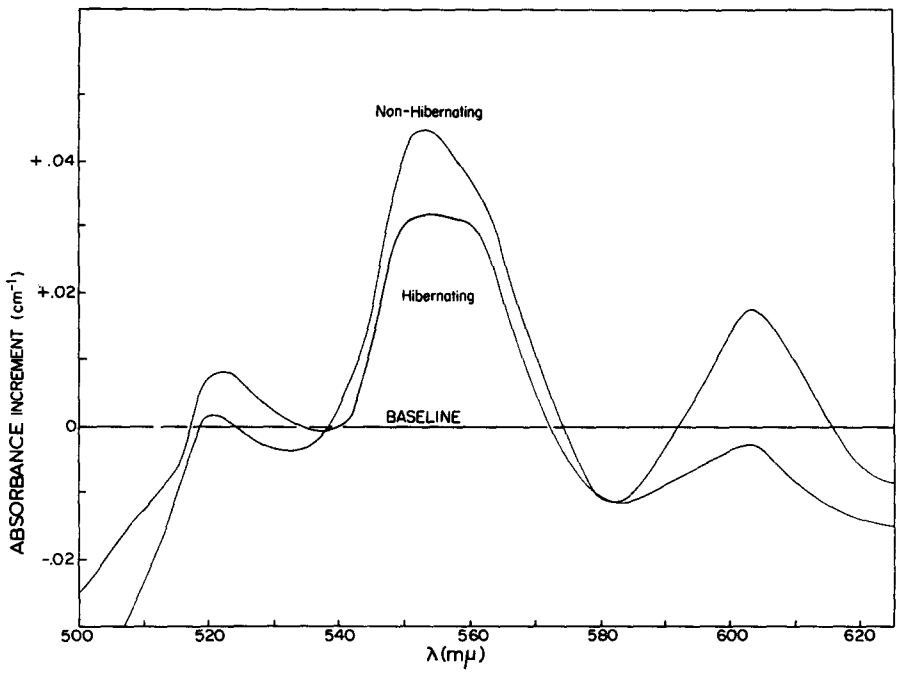

Fig. 1. Difference spectra for the respiratory components of squirrel liver mitochondria. Mitochondria of hibernator equivalent to 75 mg protein; non-hibernator equivalent to 27 mg protein. Oxidized (potassium ferricyanide) *minus* reduced (sodium hydrosulfite).

Although there does not appear to be a shift in the position of the absorption maximum of cytochromes in the hibernator, it is clear that the ratios and amounts have changed. Specifically, the peak height of c_1–c and b are equal, while that of a–a_3 is considerably decreased. Pyridine hemochromogen determinations for total mitochondrial cytochromes revealed average values for active squirrels of 3.8 nmoles/mg protein while the hibernator had an average value of only 1.5 nmoles/mg protein. Since the adaptive response of cytochromes to oxygen tension in bacteria and yeast is well known, the decrease in the pyridine hemochromogens as well as alterations in the cytochrome spectra of the hibernators might be expected. Although relatively few studies have been carried out with mammalian tissues, the induction of cytochromes in human cells by oxygen has recently been demonstrated[6].

Fig. 2 shows the oxygen electrode tracings of typical experiments in which liver mitochondria from active and hibernating squirrels were added to an aerated reaction

medium. A slow rate of oxygen uptake occurred in the presence of succinate. After addition of ADP the typical state 3,4 cycle was observed but the rate of oxygen consumption of the hibernator was only one-half that of the non-hibernator although P/O ratios were close to 2.0 in both cases. Upon addition of salicylanilide XIII, a potent uncoupler of oxidative phosphorylation[7], the respiration rate was immediately stimulated in both preparations. Surprisingly, however, the rate of the hibernator was twice that of the non-hibernator in the presence of uncoupler. The lower rate of oxygen consumption in response to ADP might be expected in view of the overall decrease in metabolism of the hibernator. The marked stimulation by uncoupler is more difficult to interpret. The respiration rate appears not to be limited by amount of cytochrome, although the hibernator contains only one-half that of the active animal. Respiration may be affected either by the penetration of nucleotides or alteration of the coupling mechanism. In connection with the former possibility, WOJTCZAK AND ZALUSKA[8] have observed that fatty acids inhibit translocation of adenine nucleotides through mitochondrial membranes. These observations may be pertinent to this study since there would tend to be considerable fatty acid oxidation in the liver during hibernation.

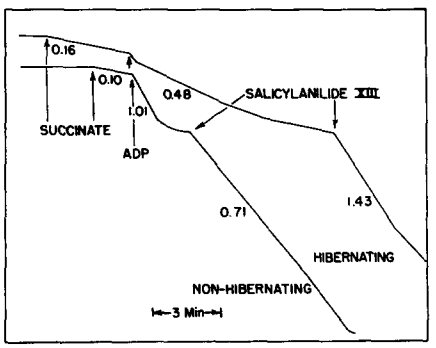

Fig. 2. Control of respiration and response to salicylanilide XIII by liver mitochondria from hibernating and non-hibernating ground squirrels. Liver mitochondria (7.0 mg protein) was added to 3.0 ml reaction mixture containing 0.17 M KCl, 0.33 M sucrose, 0.017 M KH_2PO_4, 0.01 M $MgCl_2$, and 0.01 M triethanolamine–HCl, pH 7.4. At the points indicated, succinate, 0.17 M, was added followed by the additions of ADP, $2 \cdot 10^{-4}$ M, and salicylanilide XIII, $2 \cdot 10^{-7}$ M. The numbers in the O_2 tracing represent the respiration rate expressed in μmoles of oxygen per mg protein per h. The oxygen concentration at the time of the succinate addition was $2.4 \cdot 10^{-7}$ M.

In the active state, hibernators respond to low temperature with a general increase in oxidative capacity of the mitochondria. During hibernation, however, liver mitochondria show decreases in the rates of oxidation and dehydrogenation of succinate[1]. These findings are consistent with results shown here and indicate that changes occurring during hibernation are not induced by temperature alone. The relationship of other factors, such as relative hypoxia and the nutritional status of the animal, to the mitochondrial changes remains to be evaluated. Preliminary studies in this laboratory with the diabetic rat, a highly gluconeogenic animal, have revealed mitochondrial changes somewhat similar to those observed in the hibernator. A possible relationship between electron transport and gluconeogenesis is under active investigation.

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