Changes in mitochondrial components of hamster brown adipose tissue in response to cold acclimation

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Cold acclimation of hamsters was found to result in an increase in cytochrome concentration relative to ATPase activity in brown adipose tissue mitochondria, but not in liver mitochondria. These data lend support to the hypothesis that the uncoupled respiration characteristic of non-shivering thermogenesis is the result of an adaptive change in the composition of brown adipose tissue mitochondria in response to cold acclimation.

Isolated brown adipose tissue mitochondria have been found to differ in several respects from liver or heart mitochondria. Among the most prominent differences are the relative amounts of cytochromes and mitochondrial ATPase (F_1 ATPase, EC 3.6.1.3) found in these tissues. Mitochondria isolated from mammalian liver and cardiac tissues have relatively constant stoichiometric relationships of cytochromes, F_1 ATPase and adenine nucleotide translocase (Weidemann *et al.*, 1970; Bertina *et al.*, 1973). These stoichiometric relationships are in keeping with the widely held idea that the components of the electron transport chain, mitochondrial metabolite translocation and coupling factor F_1 , are in a close physical, as well as functional, relationship (Klingenberg, 1970; Vignais, 1976). However, this situation differs in mitochondria isolated from the brown adipose tissue of hamsters. Houstek *et al.* (1978) found a difference in the ratio of cytochrome *a* to oligomycin-sensitive ATPase between mitochondria isolated from rat liver and cold-acclimated hamster brown adipose tissues, with the cytochrome *a*/ATPase ratio about 8-fold higher in brown adipose tissue. Using antimycin A to inhibit incorporation of $^{32}P_i$ into ATP, Cannon & Vogel (1977) found that hamster brown adipose tissue mitochondria required a five-fold higher titre of antimycin A to cause inhibition of respiration than did rat liver mitochondria, and that a 50% inhibition of ATP synthesis by either aurovertin or oligomycin took only one-third of the inhibitor in hamster brown adipose tissue mitochondria as in rat liver mitochondria. These data suggest that the elements of the electron transport chain are present far in excess of F_1 ATPase in hamster brown adipose tissue mitochondria when compared with mitochondria isolated from rat liver.

The present study was undertaken to determine whether the difference in the ratio of cytochrome concentration to F_1 ATPase between liver and brown adipose tissue shows adaptive changes in response to cold acclimation. The physiological responses of brown adipose tissue to cold acclimation include increased respiratory capacity, decreased P/O ratios and decreased respiratory control ratios with respect to ADP (Flatmark & Pedersen, 1975). Thus, if the greater electron transport capacity relative to coupling factor is related to the thermogenic function of brown adipose tissue, then upon cold acclimation the concentration of the electron transport system components might be expected to increase relative to coupling factor F_1 ATPase.

Experimental

Adult female hamsters, *Mesocricetus auratus* (Engle Laboratory Animals, Inc.), were provided with food and water *ad libitum*. Cold acclimated individuals were kept for at least 3 weeks prior to use at 4°C with 8 h of light and 16 h of darkness per day. Control (warm-acclimated) animals were maintained at 22°C in a natural photoperiod, approx. 11 h of daylight and 13 h of darkness (the months of February and March 1978, Ann Arbor, MI, U.S.A.) for the same period. Liver and brown adipose tissue mitochondria were isolated by the method of Johnson & Lardy (1967) and were assayed for F_1 ATPase and cytochrome content on the same day. Mitochondrial F_1 ATPase activity was assayed at 37°C in a medium containing 50 mM-Tris/HCl (pH 7.4)/4 mM-MgCl₂/50 mM-KCl/5 mM-ATP and, when specified, 0.45 mM-2,4-dinitrophenol. The reaction was initiated by adding approx. 1 mg of

mitochondrial protein. Inorganic phosphate was determined by the method of Le Bel *et al.* (1978). Absorbance at 870nm was measured on a Zeiss PMQ3 spectrophotometer. Cytochrome concentrations were determined by the method of Williams (1964) with the Zeiss PMQ3. It was necessary to pool samples from each experimental group in order to have enough tissue for accurate cytochrome determinations. Therefore the results for cytochrome could not be treated statistically. Protein was determined by the method of Lowry *et al.* (1951) with lyophilized fatty acid-free bovine serum albumin (Sigma) as a standard. Absorbance at 700nm was measured on a Coleman Junior spectrophotometer.

Results and discussion

No change was observed in brown adipose tissue mass or in mitochondrial protein yield either from brown adipose tissue or from liver in response to cold acclimation. Similarly, cold acclimation did not result in any change in F_1 ATPase activity in mitochondria isolated from liver or brown adipose tissue when assayed either in the presence or absence of dinitrophenol (Table 1). The addition of dinitrophenol to the reaction medium produced a significant ($P < 0.05$, warm acclimated; $P < 0.005$,

cold acclimated, with a one-tailed t test) increase in F_1 ATPase activity in liver mitochondria; however no change occurred in F_1 ATPase activity in brown adipose tissue mitochondria in response to dinitrophenol (Table 1). This result is interesting in the light of the properties of ATP hydrolysis and dinitrophenol action in rat liver mitochondria. The free energy of ATP hydrolysis is related to the proton-motive potential difference (Δp) across the mitochondrial inner membrane (Mitchell, 1976), such that the collapse of Δp caused by dinitrophenol leads to a stimulation of ATP hydrolysis by F_1 ATPase and the release of respiratory control of the electron transport system (Mitchell, 1976). Thus, the failure of dinitrophenol to stimulate ATPase activity in brown adipose tissue mitochondria is in accord with the well known lack of respiratory control and low P/O ratio in isolated brown adipose tissue mitochondria (Flatmark & Pedersen, 1975; Nicholls, 1979).

Cytochrome concentrations in brown adipose tissue mitochondria isolated from cold-acclimated individuals were found to be 2–3-fold greater than in warm-acclimated individuals. No such co-ordinated increase was observed in liver mitochondria isolated from the same individuals (Table 2). These results are in keeping with observations of increased cytochrome content in brown adipose tissue of

Table 1. *Liver and brown adipose tissue mitochondrial F_1 ATPase activity in the presence and absence of 2,4-dinitrophenol*

Mitochondria were isolated from liver and brown adipose tissue of warm- and cold-acclimated hamsters and were assayed for ATPase activity in the presence and absence of 0.45 mM-dinitrophenol as described in the experimental section. Values are means $\pm$ S.E.M. The number in parentheses indicates the number of samples.

Source of mitochondria	Additions	Animals ...	ATPase activity [$\mu\text{mol of P}_i \cdot \text{min}^{-1} \cdot (\text{mg of mitochondrial protein})^{-1}$]	
			Warm-acclimated	Cold-acclimated
Liver	None		0.101 ± 0.039 (6)	0.099 ± 0.040 (5)
Liver	Dinitrophenol		0.251 ± 0.029 (6)	0.283 ± 0.012 (5)
Brown adipose tissue	None		0.089 ± 0.016 (6)	0.075 ± 0.006 (5)
Brown adipose tissue	Dinitrophenol		0.067 ± 0.010 (6)	0.058 ± 0.007 (5)

Table 2. *Cytochrome a, b, c_1 and c content of liver and brown adipose tissue mitochondria of warm- and cold-acclimated hamsters*

Each value represents a determination of the cytochrome content of the pooled mitochondria isolated from liver and brown adipose tissue of six warm-acclimated and five cold-acclimated hamsters. Since mitochondria from several animals were pooled to perform a single determination, statistical analysis of the results was not possible. Isolation of mitochondria and determinations of cytochromes were performed as described in the Experimental section.

Source of mitochondria	Animals acclimated to	Content (nmol/mg of mitochondrial protein) of cytochrome:			
		a	b	c_1	c
Liver	Warm	0.091	0.111	0.088	0.084
Liver	Cold	0.150	0.202	0.084	0.071
Brown adipose tissue	Warm	0.462	0.518	0.220	0.279
Brown adipose tissue	Cold	1.220	0.967	0.204	0.647

guinea pigs and rats (Flatmark & Pedersen, 1975), increased O₂ consumption (Smith *et al.*, 1966), increased levels of succinate dehydrogenase (EC 1.3.99.1) (Barnard *et al.*, 1970) and increased levels of citrate synthase (EC 4.1.3.7) and 3-hydroxyacyl CoA dehydrogenase (EC 1.1.1.35) (Wickler, 1979) found in brown adipose tissue of various cold-acclimated small mammals. Thus, an increase in cytochrome content of brown adipose tissue in response to cold acclimation is simply part of a suite of adaptations that result in the increased oxidative capacity of the tissue.

The results presented here show no change in F₁ ATPase activity despite a large increase in cytochrome concentration in brown adipose tissue mitochondria. These results are summarized in Table 3 in terms of the ratio of cytochrome *c* concentration to F₁ ATPase activity in liver and brown adipose tissue. These ratios are similar both in the presence and absence of dinitrophenol. In either case the ratio in brown adipose tissue is far greater than in liver. The ratio in liver does not change upon cold acclimation, whereas that in brown adipose tissue increases to about 2.7-fold the warm acclimated value.

The ratio of cytochrome *c* to F₁ ATPase should reflect the ratio of oxidative to phosphorylating capacity. Several studies have shown that cytochrome *c*, cytochrome *a* and a number of citric acid cycle enzymes occur in constant proportions to one another in a wide variety of tissues from a number of species (Pette *et al.*, 1962; Klingenberg & Pette, 1962; Goebell & Klingenberg, 1963). Moreover, parallel increases in cytochrome *c* and citric acid cycle enzymes occur during the adaptive response of skeletal muscle to exercise (Holloszy *et al.*, 1970) and of brown adipose tissue to cold acclimation (Wickler, 1979). Finally, the increase in oxidative capacity of brown adipose tissue in response to cold acclimation is reflected by an increase in cytochrome concentration (Flatmark & Pedersen,

1975). Therefore, cytochrome *c* content seems to be a reasonable indicator of the respiratory capacity of brown adipose tissue. F₁ ATPase activity should reflect phosphorylating capacity, since catalysis of ADP phosphorylation and of ATP hydrolysis is thought to occur at the same binding sites (Slater *et al.*, 1979). The low F₁ ATPase activity in brown adipose tissue seen in this study correlates well with electron-microscopic studies (Lindberg *et al.*, 1967) showing brown adipose tissue mitochondria inner membrane vesicles depleted in F₁ ATPase. Electrophoretic studies of mitochondrial membrane proteins and specific inhibitor studies with aurovertin and oligomycin (Houstek *et al.*, 1978) have also demonstrated the low F₁ ATPase content of brown adipose tissue mitochondria relative to that of liver mitochondria. The validity of comparison of F₁ ATPase activity between tissues is supported by the observation that the specific activity of F₁ ATPase isolated from different tissues is similar (Cannon & Vogel, 1977). Therefore, in comparison with liver, the electron-transport capacity of brown adipose tissue (as indicated by cytochrome *c* content) is present in excess of the phosphorylating capacity (as indicated by F₁ ATPase activity). The response of brown adipose tissue to cold acclimation is a further increase in electron-transport capacity, but no increase in phosphorylating capacity. It is proposed that this is a reflection of the increased respiratory capacity and decreased respiratory control and P/O ratios that have been observed in brown adipose tissue mitochondria in response to cold acclimation (Flatmark & Pedersen, 1975).

It may be concluded that the increased cytochrome concentration/F₁ ATPase ratio previously found in hamster brown adipose tissue mitochondria relative to rat liver mitochondria (Houstek *et al.*, 1978; Cannon & Vogel, 1977) is also true relative to hamster liver mitochondria. This ratio was found to increase in brown adipose tissue mitochondria upon cold acclimation, but remained unchanged in liver mitochondria. The increase upon cold acclimation indicates that the high cytochrome concentration relative to F₁ ATPase in brown adipose tissue mitochondria is not only a feature of that tissue but that it is also related to the response of brown adipose tissue to cold acclimation.

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Table 3. Ratio of cytochrome *c* concentration to F₁ ATPase activity in liver and brown adipose tissue mitochondria

Cytochrome *c* concentration is expressed as nmol of cytochrome *c*/mg of mitochondrial protein. F₁ ATPase activity is expressed as μmol of P_i·min⁻¹·(mg of mitochondrial protein)⁻¹. All procedures are described in the Experimental section.

Source of mitochondria	Animals acclimated to	[Cytochrome <i>c</i>]/ATPase activity
Liver	Warm	0.83
Liver	Cold	0.72
Brown adipose tissue	Warm	3.14
Brown adipose tissue	Cold	8.63

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