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Decrease of oxidative activities in brown adipose tissue mitochondria of cold acclimated rats on short term exposure to heat stress

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Exposure of rats to the cold (4–5 °C) caused large (2–3-fold) increases in the mass of interscapular brown adipose tissue (BAT), its mitochondrial content and the basal metabolic rate of the animals. The rate of substrate oxidation by BAT mitochondria also increased about 3-fold. When cold-acclimated animals were exposed to heat (37 °C), the BMR decreased by half in 3 h, the earliest time interval tested. Mitochondrial substrate oxidation, as well as substrate-dependent H_2O_2 generation, showed a proportionate decrease in rates. In these mitochondria, activities of cytochrome *c* reductases, but not dehydrogenases with NADH, α-glycerophosphate and succinate as substrates, also showed a significant decrease. The concentration of cytochromes aa_3 and *b*, but not cytochrome *c*, also decreased in BAT mitochondria from 12-h heat-exposed animals, while the change in concentration of cytochrome *b* alone was found as early as 3 h of heat exposure. These results identify the change in cytochromes as a mechanism of regulation of oxidative activities in BAT mitochondria under conditions of acute heat stress.

Introduction

Exposure of endotherms to altered environmental temperatures set in motion metabolic changes, which would help in meeting the new demands and countering the adverse effects. For example, chronic exposure to the cold necessitates large thermal output to maintain the wide positive temperature differential between the body and the environment. Regulatory nonshivering thermogenesis [1] is characterized by increased basal metabolic rate and food consumption. Brown adipose tissue (BAT), which probably enjoys the highest rate of aerobic metabolism among mammalian tissues, is believed to be the primary generator of energy in nonshivering thermogenesis [2,3]. Considerable support for this view was lent by the observation that mitochondrial and cellular proliferation occurs in BAT on chronic exposure of animals to the cold, and that the stimulatory response (O_2 uptake) evoked in BAT by the thermogenic hormone norepinephrine is higher than in any other tissue [4]. The discovery of thermogenin, [5] and the elucidation of its role in BAT mitochondria as a purine nucleotide-regulated, proton-translocating uncoupler protein [6–8], led to the concept that the thermogenic function of BAT was regulated by the concentrations of purine nucleotides and thermogenin in the tissue. This view was strengthened by the increase in thermogenin concentration on treatment with norepinephrine, feeding with a high calorie diet and acclimation to cold as well as the decrease on reacclimation to heat [4,7–9]. In our view, the wide acceptance of thermogenin mediation in thermogenicity has precluded studies on other possible mechanisms of heat production by BAT mitochondria. For example, little is known about the regulation of the rate of mitochondrial electron transport in BAT by environmental temperature. We have employed the 'cold acclimation-heat exposure' approach to elucidate the phenomenon. The data presented in this paper give a clear indication that the regulation of BAT electron transport was obtained through changes in activities of cytochromes under conditions of rapid change to heat stress of cold-acclimated rats.

Abbreviations: BAT, brown adipose tissue; BMR, basal metabolic rate; RCI, respiratory control index; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; BSA, bovine serum albumin.

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Materials and Methods

Animals and treatment. Male albino rats (80–90 g) of the Wistar strain obtained from the Central Animal Facility of this institute, and maintained on a vegetable oil-based commercial (Lipton) pellet diet, were kept at 4–5 °C for 30–35 days for cold acclimation in a well-ventilated chamber. In the 'temperature shift' studies, cold-acclimated animals were transferred to another chamber (37 ± 1 °C) and kept in it for the time period indicated. Animals kept at ambient temperatures (22–25 °C) were also used as control where indicated. The rooms and chambers housing the animals were artificially lit from 9 : 00 a.m. to 5 : 00 p.m. Food and water were given ad libitum.

Basal metabolic rate. The consumption of oxygen by the whole animal was determined with the aid of an airtight, blackened desiccator to which a manometer and volume syringe (to pump air) were attached. The equipment was equilibrated at the temperature of measurement (ambient, cold or hot). (Sodium hydroxide pellets kept in a wet cloth were used to absorb the CO_2 liberated.) The animal takes a few minutes to get adjusted in the desiccator without agitation. Oxygen uptake by the animal was recorded as the time taken to absorb 10 ml of air. Four independent readings were taken for each animal and the last three values which were consistent were taken to obtain mean values and to calculate ml of oxygen consumed/min per rat at STP.

Mitochondria. Animals were killed by stunning and decapitation. Interscapular BAT was excised, freed of adhering skeletal muscle, weighed and homogenized in 0.25 M sucrose, which was filtered through double-layered cheese cloth to remove the fat. When the tissue weight was low (ambient temperature) BAT from 7 to 8 animals were pooled. At this stage, BSA (2 mg/ml) was added to bind fatty acyl CoA known to be present in this tissue. The mitochondria were then prepared by using a differential centrifugation method. The sediment was washed with 0.25 M sucrose and resuspended in the same medium at a concentration of 10 mg/ml [10]. Liver mitochondria were isolated according to Kurup et al. [11].

Enzyme assays. Mitochondrial oxygen uptake was determined by polarography using a Clark-type oxygen electrode in a Gilson Model K-ICT-C Oxygraph. The reaction system contained 50 mM sucrose, 20 mM Tris-HCl buffer (pH 7.2), 10 mM potassium phosphate buffer (pH 7.2), 2 mM $MgCl_2$, 1 mM EDTA, 1 mM KCl and about 1 mg of mitochondrial protein in a total reaction vol. of 1.4 ml. After equilibration at 30 °C, the reaction was started by the addition of 10.7 mM α-glycerophosphate, succinate or 4.5 mM ascorbate + 130 μM TMPD. When liver mitochondria were used, state 3 respiration was initiated by the addition of 215 μM ADP [12]. Respiratory control index (RCI) was calculated as the ratio of the rate of oxygen uptake in state 3 to that in state 4 (ADP exhausted; see Ref. 12). The activities of succinate dehydrogenase (succinate: (acceptor) oxidoreductase, EC 1.3.99.1) and α-glycerophosphate dehydrogenase (α-glycerophosphate: (acceptor) oxidoreductase, EC 1.1.99.5) were assayed using phenazine methosulphate + 2,6-dichlorophenol indophenol [13] and that of NADH dehydrogenase using $K_3Fe(CN)_6$ as an electron acceptor [14]. Substrate-dependent cytochrome *c* reduction was monitored by the absorbance increase at 550 nm. The rate of generation of H_2O_2 was measured by the decrease in scopoletin fluorescence as described [15]. The NADH-dependent reduction of cytochrome *c* was found to be completely inhibited on adding rotenone (1 μM) in these mitochondria.

Other estimations. The cytochrome content of mitochondria was determined from difference (reduced minus oxidized) spectra, $Na_2S_2O_4$ and ascorbate + TMPD being used for reduction [16]. The wavelength pairs and molar extinction coefficients were taken from Sundin and Cannon [17]. Cytochrome content in the homogenates was determined by pyridine hemochrome formation [32]. The reduction of cytochromes aa_3, *b* and *c* were recorded at 605–625, 562–575 and 550–540 nm, respectively, in a Hitachi 557 recording spectrophotometer in the dual wavelength mode [18]. Protein was determined by Lowry's method [19] using BSA as standard. The concentration of ubiquinone in BAT was determined after saponification and extraction [20], by the absorbance decrease at 275 nm on reduction with $NaBH_4$ [21]. Water content of the tissue was measured by differential weighing before and after desiccation until a constant weight was obtained [31]. Total lipids in homogenates of the tissue in double distilled water were extracted according to Bligh and Dyer [36], and estimated by weighing.

Chemicals. All biochemicals were obtained from Sigma, St. Louis, MO, U.S.A. All other chemicals were of the purest grades available. All solutions were prepared in water twice distilled in an all-quartz apparatus, and adjusted to pH 7.4 before use.

Results

BAT weight and mitochondrial content. When animals (80–90 g body wt.) were exposed to the cold for 35 days, there was substantial gain in body weight (189 ± 15 g), and the BMR increased more than 2-fold. The values of BMR recorded by this method (3.2 ± 0.24 ml O_2 consumed/min per rat or approx. 16 ml O_2/min per kg wt. at ambient temperature) were comparable to those reported earlier by Rothwell and Stock [33] and by Foster and Ma [34]. Interscapular BAT increased in weight by almost 400% (Fig. 1). Increase in BAT mass

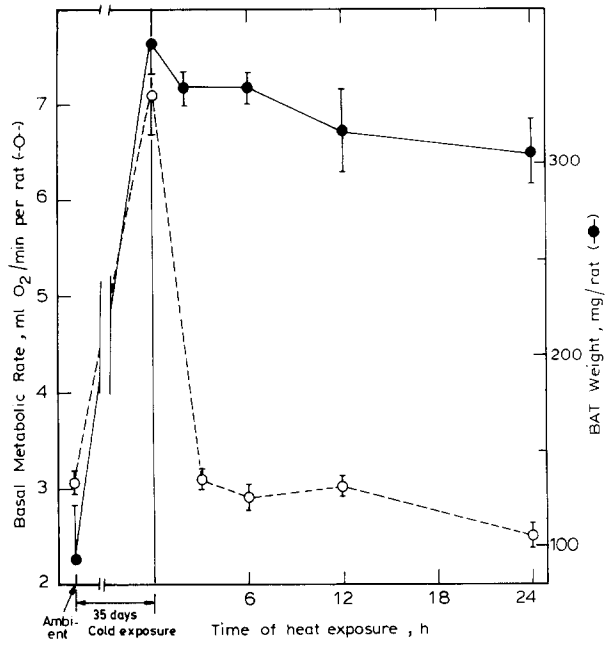

Fig. 1. Effect of environmental temperature on BAT weight and BMR of the rat. Animals were exposed to cold temperature (4–5 °C) for 35 days and then to 37 ± 1 °C for the time period indicated. The values of BMR and BAT weight are shown for animals kept at ambient temperature, exposed to the cold for 35 days and shifted to the heat (37 °C) for the time intervals (h) indicated. The mean ± S.D. of 10 animals are given.

on cold exposure is well documented and is largely caused by an increase in cell mass and not by any significant increase in cell number [22–24]. When cold-acclimated animals were exposed for as short a period as 3 h to elevated environmental temperatures (37 ± 1 °C), the BMR registered a swift and spectacular decrease, reaching normal values (ambient temperature). Tissue weight (BAT) did not show any significant decrease until 12 h ($P < 0.01$). Upon cold acclimation, the tissue protein (mg/g BAT) increases by almost 35%. At the same time, the total lipid content showed a decrease of about 40%. These results are in agreement with the previously reported values [31]. Short-term exposure of the cold-acclimated rats to 37 °C for 12 h did not cause any significant change in the total water, protein or lipid contents (Table I).

During cold acclimation the protein recovered in the mitochondrial fraction of BAT increased 2-fold (Table I). Increase in BAT mitochondria (4–6-fold) on chronic cold exposure had been observed earlier [7,25–27], and has been ascribed to increased mitochondriogenesis [27]. Exposure of cold-acclimated animals to an elevated environmental temperature (37 °C) for up to 12 h did not cause any significant change in mitochondrial content (Table I).

It may be noted that during cold acclimation the protein content of BAT registered significant increase of which mitochondrial increase accounted for only half (Table I). This would imply enhanced concentration of protein of other cellular components as well.

Oxygen uptake. BAT mitochondria isolated in our laboratory showed high rates of oxidation with a variety of substrates [15]. We also noted that the V_{max} values (nmol/min per mg protein) for α-glycerophosphate and palmitoyl CoA (without carnitine) were 260 and 84, respectively. α-Glycerophosphate dehydrogenase activity is known to increase in cold-acclimation [35] and has been postulated to be involved in the regulation of lipid synthesis and thermogenic activity. In view of these observations, we decided to study the oxidations with α-glycerophosphate as one of the substrates. During cold acclimation, the specific oxidative activity of BAT mitochondria increased significantly ($P < 0.001$). The oxidation of α-glycerophosphate was increased 4-fold and that of succinate 2-fold while the cytochrome oxidase activity increased 3-fold (Fig. 2). The increase in the rates of substrate oxidation is consistent with the increase in BMR on cold acclimation. On exposure to heat, the rate of substrate oxidation decreased by almost 50% in the initial 3 h (Fig. 2). This is in agreement with the drastic decrease in BMR reported in Fig. 1. The cytochrome oxidase activity, in contrast, decreased gradually with little change occurring in the initial 3 h of exposure.

Addition of known uncouplers like 2,4-dinitrophenol and FCCP did not further increase the oxidation rates (data not shown), indicating that the mitochondria in

TABLE I

Effect of environmental temperature on the fresh weight of BAT and some of its components

Experimental details were the same as described in the legend for Fig. 1 and Materials and Methods. Animals were cold-exposed (4 °C) for 35 days and then shifted to high temperature (37 °C) for 12 h, where mentioned. The values are the mean ± S.D. of No. of independent determinations as shown in parentheses [ ]. * $P < 0.01$; ** $P < 0.001$.

Measurements (units) [No. of determinations]	Ambient	Cold-acclimated	Heat exposed
BAT fresh wt. (mg/g BAT) [4]	95 ± 22	363 ± 49 *	328 ± 48
Water content (mg/g BAT) [4]	541 ± 72	477 ± 22	492 ± 44
Lipid content (mg/g BAT) [4]	383 ± 38	227 ± 37 *	286 ± 18
Protein content (mg/g BAT) [8]	84 ± 5	113 ± 15 **	115 ± 12
Mitochondrial protein (mg/g BAT) [16]	11 ± 2	25 ± 3 **	20 ± 3
Mitochondria based on α-glyceorphosphate dehydrogenase assay (mg protein/g BAT)	–	34 ± 5	34 ± 3

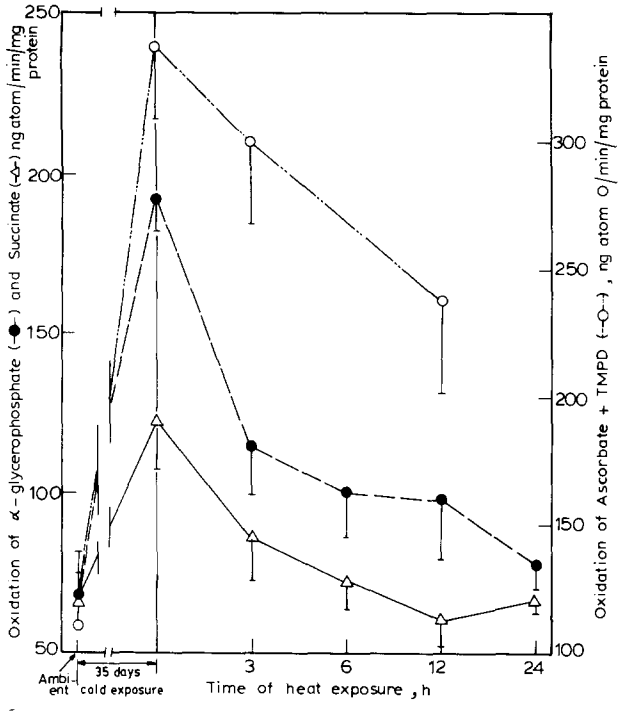

Fig. 2. Effect of exposure of the rat to cold and heat on the oxidative activity of BAT mitochondria. Cold acclimated rats (5 °C, 35 days) were exposed to heat (37 °C) for the time periods indicated. BAT mitochondria isolated from animals kept at ambient temperature, cold-acclimated and heat-exposed were assayed for the oxidation of α-glycerophosphate (--○--), succinate (-▲-) and ascorbate+TMPD (···○···). The rates of substrate oxidation by BAT mitochondria obtained from animals kept at ambient temperature, exposed to the cold for 35 days and shifted to the heat (37 °C) for the time intervals (h) indicated. The values are the mean of 6 independent samples. The mean ± S.D. are given.

our experiments are giving full respiratory capacity of the electron transport chain.

For comparison, we have measured the oxidative activity of hepatic mitochondria isolated from the same animals. Exposure to heat (37 °C) for 12 h, produced no significant change in the rate of succinate oxidation (state 3), being 203 ± 47 (heat exposed, 12 h) and 173 ± 29 (ng atom O/min per mg protein) cold acclimated (35 days), respectively. Respiratory control index also did not show any change.

Oxidoreductases. With a view to identify the specific regions, if any, of the respiratory chain sensitive to inhibition at elevated environmental temperature, the oxidoreductase activities of BAT mitochondria from cold-acclimated and heat-exposed animals were assayed. The data in Table II reveal that the membrane-bound dehydrogenases were not affected by heat exposure. In contrast, substrate dependent cytochrome *c* reduction showed a distinct and significant decrease (30–40%), which was of the same extent as the inhibition of substrate oxidation. It is interesting that a significant decrease in cytochrome *c* reductase activity occurred even at 3 h of exposure (Table II).

Reduction of cytochrome b. The data presented in Table II gave a strong indication that in the electron transport chain of BAT mitochondria, the region sensitive to inhibition on heat exposure could be the ubiquinol-cytochrome *c* segment (Complex III). Further studies on the reduction of cytochromes gave additional evidence. In this set of experiments we added substrate α-glycerophosphate to BAT mitochondria under aerobic conditions and followed the spectral changes at the appropriate wavelengths with a dual wavelength spectrophotometer to measure the reductions of the cytochromes aa_3, *b* and *c* (Fig. 3, A–C) at the onset of anaerobiosis. The tracing given in Fig. 3b indicates that the rate of reduction and the amount of cytochrome *b* were distinctly lower for BAT mitochondria obtained from cold- to heat-shifted animals. The data presented are for animals exposed for 12 h. A similar lowering of the rate of cytochrome *b* reduction was observed even after 3 h of exposure (data not shown). The time for the attainment of anaerobiosis after the addition of substrate gives a measure of the electron transport activity of the particles. In the case of mitochondria obtained from heat-exposed animals, the time is almost doubled. This is in agreement with the 50% lowering of respiratory activity shown by these preparations (Table II).

The data in this figure show another interesting feature. In BAT mitochondria from heat-exposed animals, the rate at which cytochrome *b* is reduced (absorbance change/min per mg protein) at the onset of anaerobiosis, given as numbers in parentheses, show considerable decrease in the initial phase. These results

TABLE II

Effect of exposure of cold-acclimated animals to heat on the oxidoreductase activities of BAT mitochondria

Cold acclimated animals were exposed to heat (37 °C) for the time period indicated. Other experimental details have been given in the Materials and Methods section. The enzyme activities represent nmol of electron acceptor reduced ($K_3Fe(CN)_6$, 2,6-dichlorophenolindophenol or cytochrome *c*) per min/mg mitochondrial protein and are the mean ± S.D. of 4–5 samples. * $P < 0.025$; ** $P < 0.01$.

Oxidoreductase activity	Cold-acclimated	Heat-exposed	
		3 h	12 h
NADH dehydrogenase	1820 ± 25	–	1605 ± 44
Succinate dehydrogenase	182 ± 30	–	163 ± 15
α-Glycerophosphate dehydrogenase	374 ± 32	–	375 ± 43
NADH-cytochrome *c* reductase	257 ± 44	174 ± 8**	153 ± 7**
Succinate-cytochrome *c* reductase	266 ± 36	209 ± 8*	196 ± 30**
α-Glycerophosphate-cytochrome *c* reductase	179 ± 36	105 ± 13**	113 ± 11**

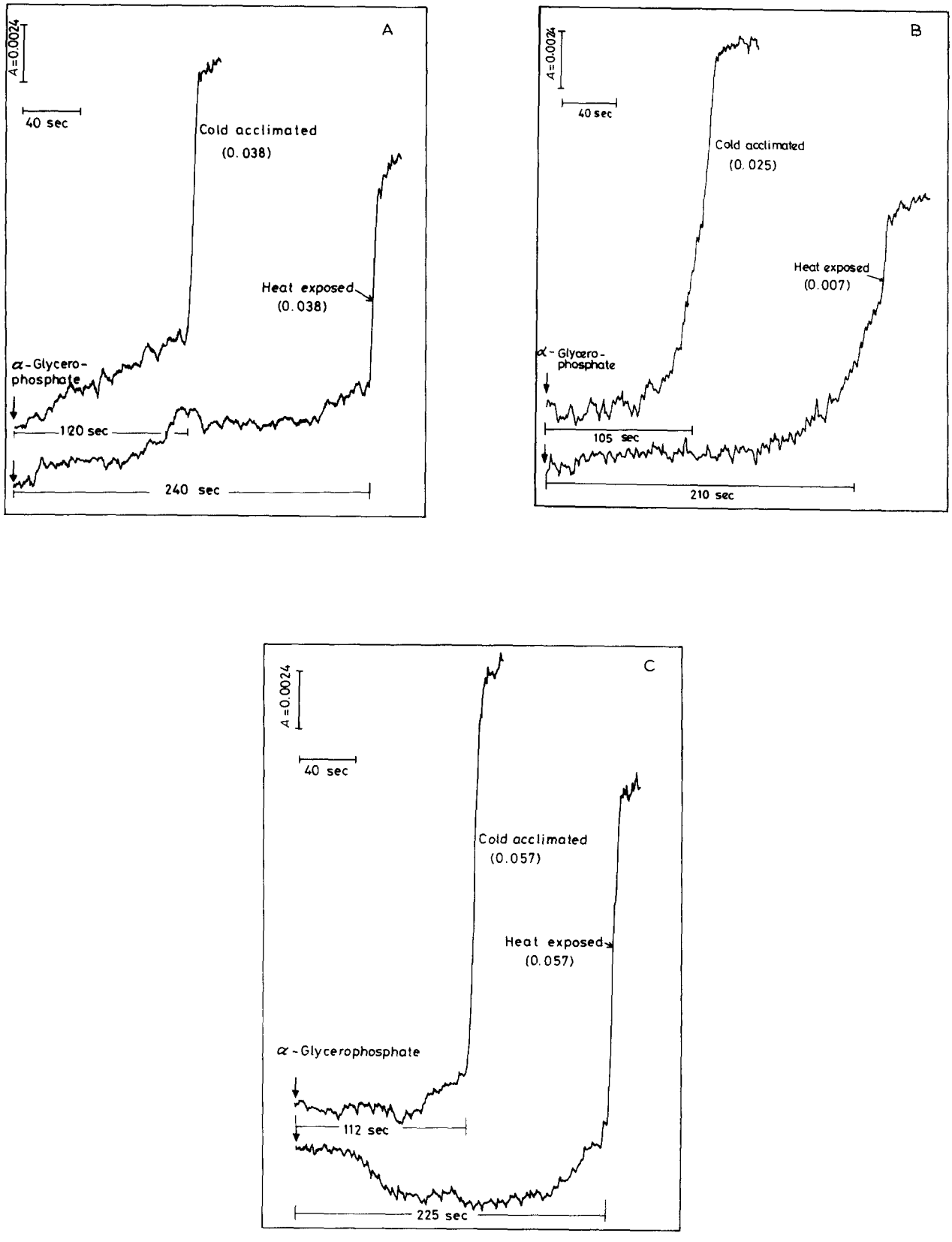

Fig. 3. Effect of exposure of cold-acclimated rats to heat on the rate of reduction of cytochromes in BAT mitochondria. Substrate (α-glycerophosphate)-dependent reduction of cytochromes in BAT mitochondria isolated after 12 h of exposure of animals to heat (37 °C) and from cold-acclimated animals was followed at respective wavelengths in a dual wavelength spectrophotometer: 3A, cytochrome aa_3, 605 nm vs. 625 nm; 3B, cytochrome *b*, 562 nm vs. 575 nm; 3C, cytochrome *c*, 550 nm vs. 540 nm. The system contained the same amount of mitochondria (1 mg), in 1 ml. The time for the onset of anaerobiosis on addition of substrate is indicated. The numbers in the parenthesis indicate the increase in the rate of absorbance at the onset of anaerobiosis expressed as absorbance change/min per mg mitochondrial protein.

TABLE III

Effect of exposure of cold-acclimated animals to heat on the cytochrome and ubiquinone content of BAT

Cold-acclimated animals were exposed to heat (37 °C) for the time period indicated. The cytochrome content of BAT mitochondria was calculated from difference spectra and in the tissue by pyridine hemochrome formation. The ubiquinone content in BAT was measured by absorbance change at 275 nm on addition of $NaBH_4$ to non-saponifiable lipids. The values given are mean ± S.D. of independent determinations (sample number: cytochromes by difference spectra, 10–13; cytochromes by pyridine hemochrome, 3; ubiquinone, 3–5). * $P < 0.05$, ** $P < 0.01$, heat-exposed vs. cold-acclimated.

Component	Method of measurement and units	Cold-acclimated	Heat exposed	
			3 h	12 h
Cytochromes	Difference spectra (pmol/mg mitochondrial protein)			
aa_3		492 ± 47	513 ± 8	369 ± 63 **
b		487 ± 59	367 ± 36 **	343 ± 34 **
c		497 ± 81	451 ± 16	466 ± 88
Cytochromes	Pyridine hemochrome (nmol/g BAT)			
aa_3		16 ± 2.0	–	12 ± 2.0 *
b		14 ± 0.3	–	10 ± 1.0 **
c		19 ± 2.0	—	18 ± 3.0
Ubiquinone	Absorbance change at 275 nm (nmol/g BAT)	175 ± 27	–	176 ± 15

are suggestive of decreased redox activity at the centre. Similar calculations carried out with cytochromes aa_3 and *c* (Fig. 3A and Fig. 3C) show that the time taken for the attainment of anaerobiosis is doubled for BAT mitochondria from heat-exposed animals, but the rate of reduction of these cytochromes at the onset of anaerobiosis does not shown any significant change, unlike that of cytochrome *b*.

The absorbance increase (562–575 nm) on the attainment of anaerobiosis indicated a strong possibility that BAT mitochondria obtained from heat-exposed animals contained less amounts of cytochrome *b*. To confirm this, the cytochrome content of the preparation was measured from difference spectra (reduced-oxidized). The values presented in Table III clearly show that exposure to heat causes a rapid and significant decrease in the content of cytochromes, especially that of cytochrome *b*. The concentration of cytochrome *b* decreased by 25% in 3 h and by 30% in 12 h and that of cytochrome aa_3, but not *c*, decreased by 25% in 12 h of heat exposure. Thus, only cytochrome *b* showed a 25% significant change as early as 3 h of heat exposure. To provide conclusive evidence for the decrease observed in cytochrome content and that it is not related to a change in recovery or sedimentation properties, we estimated the total cytochrome content present in the tissue by pyridine hemochrome formation as described earlier [32]. The values presented in Table III show a 25% decrease in cytochrome aa_3 and 30% decrease in cytochrome *b* content in 12-h heat-exposed animals. In view of this, it was of interest to study the concentration of ubiquinone, known to participate in the electron transport activity in bc_1 region. It had been reported that the electron transport activity as well as ubiquinone content were decreased in muscle mitochondria under conditions of Kearns-Sayer syndrome, mitochondrial myopathy and encephalopathy [28]. We found no change in the concentration of ubiquinone in BAT of cold-acclimated animals and those exposed to 37 °C for 12 h (Table III). This also points out that changes in cytochrome *b* are possibly responsible for the decrease in reductase activities with different substrates.

H_2O_2 generation. It is becoming increasingly evident that membrane-associated H_2O_2 generators may have a role in cellular thermogenesis [29]. It has been reported recently [15] that BAT mitochondria generated H_2O_2 more actively than did liver mitochondria. In light of this, it was of interest to see the effect of cold to heat transition on the rate of generation of H_2O_2. The data presented in Table IV give clear indication that ex-

TABLE IV

Effect of environmental temperature on H_2O_2 generation by BAT mitochondria

Cold-acclimated animals were exposed to heat (37 °C) for the period indicated. The rate of H_2O_2 generation was determined by the decrease in fluorescence of scopoletin [15]. The values are mean ± S.D. of 5 samples. * $P < 0.025$; ** $P < 0.01$.

Substrate	pmol/min per mg protein		
	cold-acclimated	heat-exposed	
		3 h	12 h
α-Glycerophosphate	240 ± 23	127 ± 21 **	91 ± 14 **
Succinate	119 ± 12	78 ± 21 *	74 ± 12 **

posure of cold-acclimated animals to heat for 3 h caused a significant (approx. 50%) decrease in the rate of generation of H_2O_2 by BAT mitochondria. In 12 h, the activity decreased by more than 60%. H_2O_2 generation had been demonstrated in several mitochondria and the rate of reaction was found to increase on addition of antimycin-A [37]. This suggests that the redox component on the substrate side of antimycin-A-sensitive site – cytochrome *b*, ubiquinone, nonheme iron proteins and flavoproteins – will act as electron donors for reduction of oxygen. Earlier studies on inhibition by metal ions and phenolic compounds [38] indicated that the sensitive component is neither the dehydrogenase or superoxide dismutase. Further, it was also shown that ubiquinone has a role in mitochondrial H_2O_2 generation [39,40] and it appears that transfer of electrons from ubisemiquinone through Fe-S center is essential for this reaction [41,15].

In the present experiments we found no change in the concentration of ubiquinone (Table III) or the rates of the dehydrogenases (Table II), implying that these parameters are not directly responsible for the decreased H_2O_2 generation during heat exposure. Proposals on implications of cytochrome *b* in H_2O_2 generation were indeed made earlier [42]. The decreases of both cytochrome *b* (Table III) and H_2O_2 generation (Table IV) after only 3 h of heat exposure, when no other component tested had changed, prompts the possibility that cytochrome *b* has a direct role in H_2O_2 generation in BAT mitochondria.

TABLE V

Contribution of BAT to basal metabolic rate in cold-acclimated and heat-exposed rats

The calculations made are based on the data presented in Table I and Figs. 1 and 2. For clarity the mean of 6–10 experiments are given.

Parameter	Control (cold-acclimated, 35 days)	Heat-exposed (cold-acclimated animals transferred to 37 °C, 3 h)
BAT wt. (mg)	363	339
Mitochondrial protein (mg/rat)	12.3	11.5
Oxygen uptake with α-glycerophosphate as substrate at 37 °C		
Specific activity (ng O atom/min per mg protein)	223	134
Total O_2 consumption (μg O atom/min)	2.74	1.54
BMR (μg O atom/min per rat)	652	295
Contribution of BAT to BMR (%)	0.42	0.52

Discussion

The results presented in this paper lend apparent support to the widely accepted view that BAT plays a role in nonshivering thermogenesis [7]. The large increases in the weight of the tissue, mitochondrial content and the rates of substrate oxidation and H_2O_2 generation observed during cold acclimation are expected responses of a thermogenic tissue. The good correlation obtained between the decrease in BMR (55%) and in total oxygen consumption by BAT mitochondria (44%) on exposure of cold-acclimated animals to heat, offer additional support. Despite these correlations, the net energy contribution by BAT under thermogenic conditions does not appear to be significant due to the smallness of the tissue. Thus, our calculations presented in Table V indicate that interscapular BAT may not account for even 0.5% of the total oxgyen consumption in cold-acclimated rats. On exposure of such animals to heat (3 h) the total oxygen uptake decreased by 357 ng atom O/min. Of this, BAT accounted for only 1.2 ng atoms (0.33%). The total mass of BAT in the cold-acclimated rat is considered to be about 5% of body weight of which interscapular tissue is about half. Based on this, the contribution from the total BAT to oxygen uptake will not be more than 1%. From the data of Foster and Frydman [30] on blood flow and arterial-venous oxygen difference of cold-acclimated animals at rest, it may be computed that only 0.7% of the total oxygen consumption is accounted for by interscapular BAT. Recently, Foster and Ma [34] have carried out extensive studies and calculations to come to the conclusion that in diet-induced thermogenesis (DIT), the contribution of BAT, which shows an increase in weight to some extent, is of the order of 2–3%. Calculations made from our data point out that contribution of BAT to the overall heat produced is small. Yet we cannot underestimate the importance of BAT in the process of heat production in view of its increased mass and other thermogenic features [2–4] obtained under all thermogenic conditions. Indeed, the main function of BAT may be to provide heat locally to important organs.

The main thrust of this work is to find rapid changes that occur on short-term exposure of cold-acclimated rats to heat. The difference in the environmental temperature is thus large (4–37 °C) and therefore, constitutes an acute stress condition. We expected that those systems that are directly involved in thermogenesis would show rapid changes. One important observation was that gross changes in weight or protein and lipid contents of the tissue or of mitochondrial protein were not obtained in view of the short term of this exposure. The significance of the decreases of total respiratory activity and of cytochromes become prominent. The findings that the activity at the level of dehydrogenases and concentrations of some of the respiratory chain components had not changed, pointed out that the brown adipose cell is responding by a

decrease of the cytochromes in order to reduce the oxidation rates to the required levels under the heat-exposure conditions. We have similar observations with respect to specific decreases in cytochrome aa_3 in liver and cytochrome *c* in kidney in rats subjected to heat stress for several days [43–45]. Cytochrome *b* in BAT was reported by Pederson and Flatmark [46] to increase by 29% on re-adapting to ambient conditions (22–23° C, 7 days) of cold-stressed (5° C, 6 days) guinea-pigs. Their contrasting results may be due to the different animal species used, or that their experimental conditions were designed to relieve from cold stress rather than to impose heat stress.

In the present experiments, changes in cytochrome *b* are found even at 3 h exposure to heat at which time other cytochromes remain unaffected. It may be stated that cold acclimation does not cause significant change in the cytochrome content of BAT mitochondria [17]. Therefore, it appears that cytochrome *b* is a target for the heat-stress response in brown adipose cell.

The data presented in this paper assume importance from another point of view also. For the first time, evidence is presented here that other mechanisms also operate in BAT for the alterations of respiratory chain-linked electron transport activities. The content of cytochrome *b* decreases significantly in 3 h of heat exposure in contrast to no change found in the content of cytochromes aa_3 and *c* in 3 h exposure. Most important, the absorbance change (0.007 absorbance change/min per mg) seen at the onset of anaerobiosis for cytochrome *b* from BAT mitochondria of heat exposed animals is markedly lower (by 70%) than for BAT mitochondria from cold-acclimated animals (0.025 Abs. change/min per mg). Such an effect of change in the rate of absorbance at the onset of anaerobiosis is not seen for cytochromes aa_3 and *c*. Therefore, we feel that cytochrome *b* could also play a role in the regulation of respiratory chain-linked electron transport activity in BAT. It may be recalled that this cytochrome is the only oxidation-reduction component entirely coded for and synthesized by the mitochondrial genome. Autonomous regulation of its levels in mitochondria of BAT would presumably be easier for the rapid response needed in such swift changes in environmental temperature.

Our results, therefore, point to the existence of other mechanisms of rapid regulation of oxidative activity, which possibly act in addition to the slow adaptation processes.

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