SHORT COMMUNICATIONS

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Oxidative phosphorylation in brown adipose mitochondria

SMITH, ROBERTS AND HITTELMAN[1] have reported that mitochondria from brown adipose tissue of cold-acclimated rats are not capable of electron-transport-linked phosphorylation but catalyze substrate-level phosphorylation. They proposed that this tissue may, therefore, have a specialized role in thermogenesis during cold adaptation and hibernation. Independent investigation in the laboratory of LINDBERG[2] yielded similar results.

These considerations allowed for the possibility that brown adipose mitochondria might represent a case of deficiency *in vivo* of one of the specific phosphorylating coupling factors. This notion was supported by the observations of a low ATPase activity in these mitochondria[1] and of a deficiency in inner membrane spheres[2], suggesting that F_1 might be a limiting component[3,4].

This report presents our findings with respect to the phosphorylation efficiency and ATPase activity of brown adipose mitochondria from the hibernating gland of newborn rabbits and of brown adipose mitochondria from cold-acclimated rats.

Brown adipose mitochondria from newborn male rabbits* as well as from the brown fat gland from the intrascapula region of male white rats maintained at 0–4° for 45 days were isolated by a procedure similar to that of SMITH, ROBERTS AND HITTELMAN[1]. A minor modification of this procedure involved the passage of the washed brown fat gland through a hand-driven French press with a fine sieve prior to homogenization with a loose-fitting homogenizer. Defatted albumin was prepared by extracting crystalline bovine serum albumin or Sigma bovine serum albumin Fraction V (powdered) with 95 % ethanol until the extracts were essentially free of acid. The defatted albumin was then dialyzed against distilled water.

ATPase was determined as described previously[3]. Mitochondrial protein was determined by the biuret method after solubilization with deoxycholate[5]. Oxidative phosphorylation was measured as described by FESSENDEN AND RACKER[6].

Oxidative phosphorylation in brown fat mitochondria

From Table I it can be seen that the rabbit and rat brown fat mitochondria oxidized α-oxoglutarate and succinate at a rate similar to that reported by SMITH, ROBERTS AND HITTELMAN[1] for rat brown fat mitochondria. Phosphorylation was negligible with succinate as substrate. With α-oxoglutarate the P:O ration was 0.89, consistent with reported values[1]. However, the addition of 5–8 mg of defatted albumin to the reaction mixture resulted in a striking restoration of phosphorylation both in the rat and rabbit brown fat mitochondria**. The P:O ratios were comparable to

Abbreviation: F_1, coupling factor 1 (ref. 4).

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** After completion of this manuscript we learned from Dr. C. D. JOEL that he has made similar observations with brown fat mitochondria from cold-acclimated rats.

those obtained with mitochondria from other tissues. Large amounts of crystalline albumin (dialyzed) also stimulated the P:O ratio but not as efficiently as the defatted preparation.

TABLE I

EFFECT OF BOVINE SERUM ALBUMIN ON OXIDATIVE PHOSPHORYLATION IN RABBIT AND RAT BROWN FAT MITOCHONDRIA

To a final volume of 0.5 ml were added: $MgSO_4$ (1.0 μmole); ATP (0.5 μmole); glucose (35 μmoles); Tris–sulphate (pH 7.4) (2.5 μmoles); EDTA (2.5 μmoles); dialyzed bovine serum albumin (0.50 mg); sucrose (5.5 μmoles); hexokinase (2.8 units); phosphate buffer (pH 7.4) (25 μmoles); $^{32}P_i$ ($2 \cdot 10^5$ counts/min); succinate (25 μmoles); or α-oxoglutarate (25 μmoles); 1.4 mg rabbit brown fat mitochondria or 1.0 mg rat brown fat mitochondria. After shaking for 24 min (rabbit) and 30 min (rat) in a Warburg apparatus at 30° a deproteinized sample of the reaction mixture was analyzed as described previously[6].

Source	*Additions to brown fat mitochondria*	*O_2 uptake (μatoms)*	*Glc-6-^{32}P formation (μmoles)*	*P:O ratio*
Rabbit	Succinate	2.11	0.203	0.096
	Succinate + 5.8 mg defatted albumin	3.73	6.2	1.66
	α-Oxoglutarate	11.7	10.4	0.89
	α-Oxoglutarate + 5.8 mg defatted albumin	6.43	17.3	2.69
Rat	Succinate	1.94	0.20	0.10
	Succinate + 7.0 mg defatted albumin	5.59	6.80	1.24

The addition of large amounts of defatted albumin also had some effect on the rate of respiration. With α-oxoglutarate as substrate addition of defatted albumin inhibited respiration, whereas it stimulated respiration with succinate. The degree of stimulation varied with the mitochondrial preparation between 1.5- to 3-fold. Increasing succinate concentration (20–100 mM), with or without the addition of defatted bovine serum albumin, increased the rate of oxidation in brown fat mitochondria maximally 3-fold without a change in the phosphate to oxygen ratio.

TABLE II

EFFECT OF INHIBITORS ON OXIDATIVE PHOSPHORYLATION OF BROWN FAT MITOCHONDRIA

Experimental conditions were as described in Table I with 1.64 mg rabbit brown fat mitochondria and succinate as substrate. Defatted albumin (7.37 mg) was added to all vessels.

Additions		*O_2 uptake (μatoms)*	*Glc-6-^{32}P formation (μmoles)*	*P:O ratio*
None		6.76	6.86	1.01
Oligomycin	(0.2 μg)	7.75	5.17	0.67
Oligomycin	(2.0 μg)	5.40	0.31	0.06
Atractyloside	(6.25 mμmoles)	6.10	1.93	0.32
Atractyloside	(12.50 mμmoles)	5.82	0.79	0.14
Dio-9	(45.0 μg)	7.65	0.01	0.0
Dio-9	(90.0 μg)	8.05	0.0	0.0

Phosphorylation stimulated by albumin with succinate as substrate was inhibited by oligomycin, atractyloside and Dio-9* (Table II). It can be seen that even low concentrations of oligomycin (0.2 μg/1.64 mg protein) depressed the P:O ratio. Oxidative phosphorylation in brown fat mitochondria was quite sensitive to Dio-9 (ref. 7). Brown fat mitochondria from rabbit tissue catalyzed a very low $^{32}P_i$–ATP exchange which was only slightly increased by the addition of bovine serum albumin.

ATPase

The ATPase activity of the brown fat mitochondria from rabbit tissue was not stimulated greatly by 2,4-dinitrophenol (Table III) nor by carbonylcyanide *m*-chloromethoxyphenylhydrazone, but was activated 2-fold by storage at —70° as a dilute suspension (11 mg protein/ml of 0.25 M sucrose). Oligomycin and Dio-9 inhibited markedly the ATPase activity of the fresh preparation but somewhat less the aged preparation. Addition of albumin (3–12 mg) had no influence on the ATPase activity of brown fat mitochondria.

TABLE III

ADENOSINE TRIPHOSPHATASE ACTIVITY OF BROWN FAT MITOCHONDRIA FROM RABBITS

Rabbit brown fat mitochondria (0.56 mg) were incubated for 10 min at 30°.

Expt. No.	*Additions*	*μmoles P_i liberated per mg per h*	
	Mitochondria:	*Freshly prepared*	*Aged at —70°*
1	None	5.49	11.2
	+ 2,4-dinitrophenol (0.2 mM)	6.84	12.9
	+ 2,4-dinitrophenol (0.6 mM)	7.11	13.4
	+ oligomycin (1 μg)	0.39	2.14
	+ oligomycin + 2,4-dinitrophenol (0.4 mM)	0.39	1.28
2	None	8.85	12.0
	+ Dio-9 (45 μg)	3.51	4.87
	+ Dio-9 (90 μg)	0.90	2.75

A soluble preparation of ATPase was obtained after shaking rabbit brown fat mitochondria with glass beads in a Nossal shaker[3] in an atmosphere of N_2. Similar to the enzyme from beef-heart mitochondria[4] this solubilized ATPase was cold-labile.

Submitochondrial particles

Submitochondrial particles prepared from rabbit brown fat mitochondria by sonic oscillation[8] had a very low P:O ratio and virtually no $^{32}P_i$–ATP exchange activity. The P:O ratio could not be increased by low levels of oligomycin, high concentrations of albumin nor by various coupling factors from beef-heart mitochondria[9]. Electron micrographs revealed somewhat irregularly shaped submitochondrial particles, some of which were lined with characteristic inner membrane spheres which were shown to catalyze in submitochondrial particles from beef heart a Mg^{2+}-dependent ATP hydrolysis[10]. In line with this finding is the presence of Mg^{2+}-dependent ATPase activity in these submitochondrial particles (Table III).

The data presented in this paper clearly demonstrate that brown rat mito-

* Dio-9 was obtained from the Royal Netherlands Fermentation Industries, Ltd., Delft, The Netherlands.

chondria of cold-adapted rats and of young rabbits are capable of oxidative phosphorylation with a P:O ratio comparable to that of other tissues. However, large amounts of defatted albumin had to be added indicating the presence of uncoupling agents in these preparations. In view of this finding and of the origin of these mitochondria, it is quite likely that they contain long-chain fatty acids which are known to be uncouplers[11].

The question arises whether the presence of these uncouplers is an artifact arising during the preparation of the mitochondria or whether fatty acids are liberated *in vivo* and thus regulate the heat production in the tissue. It is possible that such a regulatory mechanism takes place in the cold perhaps *via* hormonal control, *e.g.* norepinephrine which is known to stimulate lipolysis in brown adipose tissue[12].

A regulatory function of fatty acids in energy metabolism first proposed by PRESSMAN AND LARDY[11] has been recently documented in retrogressing mammary glands of guinea pigs[13]. It appears, however, that in the latter case the increased content in long-chain fatty acids is due to a decrease of fatty acid esterification rather than increased lipolysis (D. JONES AND W. L. NELSON, personal communication).

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