Mitochondria from Brown Adipose Tissue

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(Received 12 January 1967)

Brown fat is a tissue that is specially adapted to produce heat. It is present to an appreciable extent in newborn mammals such as rabbits and also in cold-acclimatized rats. By electron microscopy it has been shown that the cells contain large numbers of mitochondria with densely packed cristae (Lindberg, De Pierre, Rylander & Afzelius, 1967). Two papers have appeared claiming that mitochondria from brown adipose tissue of the cold-acclimatized rat (Smith, Roberts & Hittleman, 1966) and from newborn rabbits (Lindberg *et al.* 1967) do not synthesize ATP by an electron-transport-coupled system. When the present work was almost completed a preliminary statement appeared (Hohorst & Stratmann, 1967) that mitochondria from the tissue of the guinea pig do carry out oxidative phosphorylation. From the former results it has been concluded that these mitochondria are specifically adapted to produce heat without the synthesis of ATP. If this is so they might be 'uncoupled', as after treatment with 3,5-dinitro-*o*-cresol (Aldridge & Stoner, 1960). Alternatively they might not contain the enzymic systems necessary for the utilization of energy from electron transport to synthesize ATP from ADP and P_i , in which case such mitochondria would provide a valuable preparation for comparative studies with other mitochondrial preparations. We became interested in this possibility in our studies of the mechanism of action of trialkyltins on oxidative phosphorylation (Aldridge, 1958).

Rats were cold-acclimatized at 2–4° for 21–70 days (Aldridge & Stoner, 1963). Brown adipose tissue (1.5 g. from three rats) was dissected from the scapula region, separated from muscle and connective tissue and dropped into ice-cold medium. It was chopped with scissors and then homogenized in 8 ml. of 0.3 M-sucrose containing bovine serum albumin (2%, w/v) in the homogenizer previously described (Aldridge, Emery & Street, 1960; Webster & Smith, 1964) with a speed of rotation of 1900 rev./min. and 0.02 in. difference in diameter between pestle and tube (15 strokes up and down were necessary). An additional 12 ml. of sucrose-albumin solution was added and mixed by two further strokes up and down. After being poured through one layer of surgical gauze, which was gently squeezed, the solution was centrifuged at 850 g for 10 min. The supernatant was gently

poured off without disturbing fat on the wall of the centrifuge tube. Mitochondria were sedimented at 5000 g for 15 min. A fatty layer and all the supernatant were removed by suction. The inside of the tube was wiped with paper tissue to remove fat. The pellet of mitochondria was suspended in sucrose-albumin solution and again centrifuged at 5000 g for 15 min. After removal of the supernatant the pellet of mitochondria was gently resuspended and diluted to 4 ml. with sucrose-albumin solution. For some experiments the fraction sedimenting at 850 g for 10 min. was not separated (Table 1, Expts. 6–8). Brown adipose tissue from 3–5-day-old rabbits was dissected from the scapula region. For all preparations best results were obtained if they were used immediately. The following methods were used: measurement of oxidative phosphorylation (method 2 of Aldridge & Parker, 1960); assay of adenosine triphosphatase activity (Aldridge & Stoner, 1960); removal of fatty acids from albumin (Chen, 1967); determination of protein (Aldridge, 1962).

The results in Table 1 show that relatively small changes in isolation procedure alter respiration and P/O ratio of mitochondrial fractions from brown adipose tissue. There is no doubt that oxidative phosphorylation is occurring, for P/O ratios approx. 1 may be readily obtained (preparation *D*). Even without the removal of the fraction sedimenting at 850 g for 10 min. a P/O ratio 0.63 was obtained (preparation *E*). In all these experiments pyruvate was used as a substrate; the maximum P/O ratio for substrate-level phosphorylation will be 0.2. In Expts. 4–9 oxygen uptake and phosphate uptake were linear with time for 45 min. Respiration of all preparations was increased by NAD and cytochrome *c*. Phosphorylation was inhibited by 2,4-dinitrophenol (30–50 μ M; Expts. 7–9); the lower inhibition compared with that obtained with liver mitochondria (Aldridge & Street, 1964) is undoubtedly due to binding of 2,4-dinitrophenol by albumin (Teresi & Luck, 1948).

Expts. 10 and 11 show that our best method used for rat tissue (preparation *D*) is applicable to tissue from newborn rabbits (preparation *F*); oxygen uptake and phosphate uptake were linear with time, P/O ratios 1.21 and 1.44 were obtained and phosphorylation was inhibited by 2,4-dinitrophenol (Expt. 11).

Table 1. *Oxidative phosphorylation by preparations of mitochondrial fractions from brown adipose tissue*

Methods of preparation were as follows. *A*, Rat tissue was homogenized, and the fraction was washed and suspended in 0.3 M-sucrose without albumin and used after storage for 2 hr. at 0°. *B*, Rat tissue was homogenized in 0.3 M-sucrose containing crystalline bovine serum albumin (2%, w/v), and the fraction was washed and suspended in 0.3 M-sucrose without albumin and used after storage for 2 hr. at 0°. *C*, Rat tissue was homogenized, and the fraction was washed and suspended in 0.3 M-sucrose containing crystalline bovine serum albumin (2%, w/v) (the preparation was used immediately). *D*, As for preparation *C*, except that crystalline bovine serum albumin treated to remove fatty acids was used. *E*, As for preparation *D*, but the fraction sedimenting at 850g for 10 min. was not separated. *F*, Rabbit tissue, as for preparation *D*. All experiments were at 37°. Where indicated co-enzymes were added (NAD, 1.5 μ moles, and cytochrome *c*, 0.03 μ mole, in 3 ml. of medium). Except for Expt. 9 all P/O ratios were determined in the presence of NAD and cytochrome *c*. Results are expressed as means $\pm$ s.e.m. with the numbers of observations in parentheses.

Expt. no.	Method of preparation	Concn. of albumin in reaction medium (%)	Oxygen uptake (μ g.atoms/mg. of protein/min.)		P/O ratio
			Coenzymes added	No coenzymes added	
1	<i>A</i>	Nil	0.60 $\pm$ 0.11 (5)	0.34 $\pm$ 0.04 (5)	< 0.3
2	<i>B</i>	Nil	1.10 $\pm$ 0.08 (5)	0.53 $\pm$ 0.07 (5)	—
3	<i>C</i>	0.07	1.78 $\pm$ 0.06 (5)	1.39 $\pm$ 0.12 (5)	—
4	<i>D</i>	0.07	0.84 $\pm$ 0.05 (3)	—	0.78 $\pm$ 0.06 (3)
5	<i>D</i>	0.57	0.60 $\pm$ 0.04 (7)	0.32 (1)	0.92 $\pm$ 0.08 (7)
6	<i>E</i>	0.57	0.75 $\pm$ 0.04 (5)	0.27 (1)	0.63 $\pm$ 0.07 (3)
7	<i>E</i>	0.57	0.82	—	0.76
	*	0.57	0.96	—	0.56
8	<i>E</i>	0.17	0.64	—	0.52
	†	0.17	0.90	—	0.16
9	<i>D</i>	0.17	—	0.32	1.13
	†	0.17	—	0.49	0.17
10	<i>F</i>	0.57	0.70 (2)	—	1.32 (2)
11	<i>F</i>	0.17	0.71	—	1.44
	†	0.17	0.98	—	0.30

* 30 μ M-2,4-Dinitrophenol present.

† 50 μ M-2,4-Dinitrophenol present.

Under the same experimental conditions, oxygen uptake of liver mitochondria is 0.16 μ g.atom/mg. of protein/min. (Aldridge & Parker, 1960); this is not increased by the addition of NAD and cytochrome *c*. Previous published results for mitochondria from brown adipose tissue from cold-acclimatized rats are 0.11–0.17 (Smith *et al.* 1966) and from newborn rabbits 0.082 (Lindberg *et al.* 1967), both in the absence of NAD and cytochrome *c*. Even with the preparation (*D*) showing the highest P/O ratio there was considerable stimulation of oxygen uptake in the presence of NAD and cytochrome *c* (Expt. 5). We conclude that mitochondria from brown adipose tissue have a high oxidative capacity but can also synthesize ATP via an electron-transport-coupled system. Compared with mitochondrial preparations from other tissues the measured P/O ratio is low; however, they may be damaged, as suggested by the known effects of fatty acids and the improvement in the presence of albumin. The stimulation of oxygen uptake by NAD and cytochrome *c* further supported the idea

that the mitochondria were damaged, but the existence of two populations of mitochondria (one undamaged, oxygen uptake not increased by NAD and cytochrome *c* and carrying out oxidative phosphorylation; the other damaged, showing no oxygen uptake in the absence of NAD and cytochrome *c*) cannot be definitely excluded by Expt. 9.

Triethyltin (3 μ M) inhibits by 50% respiration of these mitochondria; this is a higher concentration than that required for tightly coupled liver mitochondria (0.3 μ M; Aldridge & Street, 1964), but is reminiscent of that required in early experiments with liver mitochondria possessing little respiratory control (Aldridge & Cremer, 1955).

Adenosine triphosphatase activity was measured in a few experiments [preparation *D*; 0.13 $\pm$ 0.01 (4) μ g.atom/mg. of protein/hr.]; no stimulation was obtained with 2,4-dinitrophenol, but partial inhibition was seen with 2 μ g. of oligomycin/ml. The lack of stimulation of adenosine triphosphatase activity by 2,4-dinitrophenol is difficult to explain (cf. Expts. 7–9 and 11).

Thus, though it is probably wiser at the present time to consider that our preparations are still damaged and that this is the cause of the low P/O ratio, we cannot exclude the possibility that only one of the usual three sites of synthesis of ATP is operative. Whatever are the conclusions about the properties of mitochondria from brown adipose tissue, it is clear that preparations from the rat and rabbit contain the enzymic systems necessary for oxidative phosphorylation. From this and other work (preparations from guinea pig; Hohorst & Stratmann, 1967) we consider that this conclusion is probably true for mitochondria from brown adipose tissue of other species. The inability to demonstrate elementary particles by negative-staining techniques (Lindberg *et al.* 1967) may not be correlated with an absence of phosphorylation coupled to electron transport.

We are grateful to Professor O. Lindberg for discussions and to Professor E. Racker for telling us of the importance

of rigorously removing fatty acids from crystalline bovine serum albumin.

- Aldridge, W. N. (1958). *Biochem. J.* **69**, 367.
Aldridge, W. N. (1962). *Biochem. J.* **83**, 527.
Aldridge, W. N. & Cremer, J. E. (1955). *Biochem. J.* **61**, 406.
Aldridge, W. N., Emery, R. C. & Street, B. W. (1960). *Biochem. J.* **77**, 326.
Aldridge, W. N. & Parker, V. H. (1960). *Biochem. J.* **76**, 47.
Aldridge, W. N. & Stoner, H. B. (1960). *Biochem. J.* **74**, 148.
Aldridge, W. N. & Stoner, H. B. (1963). *Biochim. biophys. Acta*, **78**, 736.
Aldridge, W. N. & Street, B. W. (1964). *Biochem. J.* **91**, 287.
Chen, R. F. (1967). *J. biol. Chem.* **242**, 173.
Hohorst, H. J. & Stratmann, D. (1967). *Abstr. 4th Meet. Fed. Europ. biochem. Soc., Oslo*, p. 109.
Lindberg, O., De Pierre, J., Rylander, E. & Afzelius, B. A. (1967). *J. Cell Biol.* **34**, 293.
Smith, R. E., Roberts, J. C. & Hittleman, K. J. (1966). *Science*, **154**, 653.
Teresi, J. D. & Luck, J. M. (1948). *J. biol. Chem.* **174**, 653.
Webster, G. R. & Smith, A. T. (1964). *Biochem. J.* **90**, 64.