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Structural Factors Involved in the Reduction of External DPN * by Mitochondrial Systems

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Previous studies [1] of the mitochondrial organization of DPN-linked oxidative systems have indicated the involvement of reversibly alterable structural factors in the availability of external DPN for intramitochondrial dehydrogenase reactions. The present report constitutes a complement to these studies, dealing with the effects of aging and subsequent structural reconstruction by ATP and Mn^{++} on the capacity of mitochondria to oxidize glutamate with external DPN as the terminal hydrogen acceptor.

Twice washed mitochondria from one rat liver, prepared in 0.25 M sucrose, were suspended in 20 ml 0.15 M KCl, containing 24 mg AMP, 174 mg K_2HPO_4 and 216 mg glucose (pH 7.5); the suspension was supplemented with 0.6 ml 0.1 M $MgCl_2$ and an excess of hexokinase, and diluted with 0.25 M sucrose to 30 ml. The suspension was aged at 30° C in a 200 ml Erlenmeyer flask under shaking in the presence of air. 0.2 ml aliquots were removed before and during aging and transferred to Beckman cuvettes containing 1.1 ml 0.05 M potassium phosphate buffer (pH 7.5), including 2.7 µmoles DPN, 1 µmole KCN and other additions to be tested. Silica plungers, reducing the light path to 1 mm, were placed in the cuvettes and optical density was measured at 340 mµ. After the zero time reading, 0.2 ml 0.1 M glutamate was added and the readings repeated each minute. The increase, proportional to the formation of DPNH, was virtually linear during the first five minutes. The blank contained all components but glutamate, which was replaced by 0.2 ml 0.05 M phosphate buffer.

In experiments entailing a determination of respiration and phosphorylation, 1.5 ml aliquots of the mitochondrial suspension were transferred to Warburg vessels and supplemented with 0.2 ml 0.1 M glutamate, as well as desired individual additions, to a final volume of 2 ml. The analytical procedures have been described elsewhere [2]. All experiments were performed at 25° C.

Table 1 shows that the rate of DPNH formation markedly increases upon aging and reaches a maximum after about 80 minutes. This increase can be prevented by Mn^{++} (Table 2), or reversed to the level prevailing in the intact system by the addition of ATP

Table 1. Effect of aging on DPNH formation. Values referred to mitochondria derived from 100 mg liver.

Aging time minutes	µmoles DPNH/5min.
0	0.68
20	1.16
40	1.33
60	1.91
80	2.32
100	2.11

Table 2. Prevention of aging effect by Mn^{++}. Time of aging: 60 minutes. Mn^{++}, 0.5 mM. Values referred to mitochondria derived from 100 mg liver.

System	µmoles DPNH/5min.
intact	0.47
aged	1.26
aged with Mn^{++}	0.49

* Abbreviations: DPN, diphosphopyridinenucleotide; DPNH, reduced diphosphopyridinenucleotide; AMP, adenosine-5-phosphate; ADP, adenosinediphosphate; ATP, adenosine triphosphate.

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Table 3. Restoration of aging effect. Time of aging: 60 minutes. Additions: AMP, ADP and ATP, 2.4 mM; Mn^{++}, 0.5 mM. Values referred to mitochondria derived from 100 mg liver.

System	Additions	μmoles DPNH/5min
intact	—	0.62
aged	—	2.31
	AMP	2.24
	ADP	1.43
	ATP	0.82
	Mn^{++}	2.35
	ATP+Mn^{++}	0.65

and Mn^{++} (Table 3). In the latter case, Mn^{++} alone is without effect, while ATP alone or, to a lesser extent, ADP, but not AMP, may exhibit a certain action.

In the experiment presented in Table 4, it is shown that the effect of ATP and Mn^{++} is not due to an inhibition of the oxidation of glutamate, but rather to a structural reconstruction of the mitochondrial system, also involving a restoration of the phosphorylative ability.

The results support the previous postulate [1] that the availability of cytoplasmic DPNH is controlled by the structural state of the mitochondria.

1. Ernster, L. *Exptl. Cell Research* **10** (1956) 704, 721.
2. Ernster, L. and Löw, H. *Exptl. Cell Research, Suppl.* **3** (1955) 133.

The Effect of Thyroxine on Mitochondrial Stability

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It has been demonstrated repeatedly that thyroxine, either administered *in vivo* [1-3] or applied *in vitro* [1,2,4-8], may lower the P/O ratio of isolated rat liver mitochondria. The action of thyroxine applied *in vitro* can be obliterated by Mg^{++}, and more efficiently [7] by Mn^{++}. These ions had been shown previously to protect mitochondria against the action of calcium [9,10]. Using phosphorylative ability [10] and optical density [11] as criteria, it has been shown that under suitable conditions the stability of the enzymic and morphological integrity of isolated mitochondria is an inverse function of the concentration ratio of Ca^{++}/Mg^{++} and/or Ca^{++}/Mn^{++} in the system. It was therefore visualized that the action of thyroxine may be reflected primarily in a labilization of mitochondria rather than in a direct action on oxidative phosphorylation. Optical density studies of liver mitochondrial preparations derived from normal and thyroxine treated rats have lent support to this concept [8]. A labilization of the system has been demonstrated to occur even at doses of thyroxine where a direct effect on oxidative phosphorylation could not be ascertained.

All rats used were of the Wistar strain weighing between 125 and 150 g. Hyperthyroidism

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Table 4. DPNH formation and oxidative phosphorylation in aged and restored systems. Additions: DPN, 1.8 mM; ATP, 2.4 mM; Mn^{++}, 0.5 mM. Values referred to mitochondria derived from 100 mg liver.

Aging time minutes	Additions	μmoles DPNH/5 min. (in presence of KCN)	μat. O_2/5 min. (in absence of KCN)	P/O
0	—		1.57	2.84
	DPN	0.88		
30	—		0.45	
	DPN	1.94	1.81	0.33
	DPN, ATP, Mn^{++}	0.69	1.35	1.65
60	—		0.12	
	DPN	2.81	1.09	0.25
	DPN, ATP, Mn^{++}	0.92	1.09	1.86