THE RESPIRATORY ACTIVITY OF ISOLATED MAMMALIAN NUCLEI. I

THE NADH OXIDASE IN RAT LIVER NUCLEI*

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Siebert (1961) has shown that non-aqueous liver nuclei contain glycolytic enzymes and malic and isocitric dehydrogenases. Nuclei of rat liver and kidney isolated in M/4 sucrose contain NADH-cytochrome c and NADH-neotetrazolium reductases, as well as succinoxidase and cytochrome oxidase (Rees and Rowland, 1961; Rees et al., 1962). Rees and Rowland assumed the succinoxidase to be mitochondrial in origin and used it as a basis to calculate the mitochondrial contribution to the other activities. Thus they found that liver nuclei have an apparent ability to oxidize NADH and reduced cytochrome c, and they postulated that nuclei possess their own electron transport system. However, nuclei are known to be capable of greatly stimulating mitochondrial oxygen uptake, substrate utilization and phosphorylation (Potter et al., 1951 and Johnson and Ackermann, 1953). Their interaction in vivo has been described also (Moses, 1964). We feel that this ability of nuclei and mitochondria to interact has been overlooked in previous studies and that, in light of this property, nuclear respiratory ability can only be assessed with validity in the absence of mitochondria.

This laboratory has been studying aerobic phosphorylations catalyzed by liver nuclei isolated in dense sucrose (Penniall et al., 1963, 1964a; Penniall and Saunders, 1964; Penniall and Griffin, 1964). The

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nuclei are devoid of mitochondria, as evidenced by the properties of the phosphorylation, and low temperature spectroscopy (Penniall et al., 1964b). Using such nuclei we have found that they have an inherent capacity to oxidize NADH. Surprisingly, we have found that maximal activity of the nuclear respiration requires the presence of a non-nuclear protein, cytochrome c.

Experimental - Liver nuclei and mitochondria were isolated simultaneously in dense sucrose from mature male Sprague-Dawley rats as described previously (Penniall et al., 1964a). NADH-neotetrazolium reductase was measured as described by Slater (1959). NADH-oxidase was measured with the Clark electrode; a GME oxygraph provided a constant voltage source and recorded changes in oxygen tension. Under the conditions employed nuclear NADH oxidation was linear with time; the cytochrome c was not autoxidizable with excess ascorbate. Protein was determined by the biuret procedure (Penniall et al., 1964a). β-NADH and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. DNA-ase was obtained from Worthington Biochemical Corp., Freehold, N. J. For electron microscope work, aliquots of nuclei were dried on agar, coated with collodion, and examined both with and without chromium shadowing using a Hardco Akashi TRS-50 electron microscope (Sharp and Beard, 1952).

Results - As expected (McEwen et al., 1963), we have found liver nuclei isolated in 2.2 M sucrose unable to oxidize a variety of NAD-linked substrates. However, in exploratory experiments nuclei did exhibit an ability to oxidize NADH with neotetrazolium (NTZ) as an electron acceptor. Table I presents an experiment wherein nuclei show NADH-NTZ reductase, but not succinic-NTZ reductase. It shows also that mitochondria retain succinic-NTZ reductase activity; and that, were the NADH-NTZ reductase of the nuclei due to contaminating mitochondria, then the nuclei should have detectable succinic-NTZ reductase.

Table I

NADH-Neotetrazolium Reductase of Liver Nuclei and Mitochondria

Conditions: 1.0 mg. nuclear protein or 1.3 mg. mitochondrial protein; 100 µmoles Pi, pH 7.4; 1.1 µmoles NADH or 20 µmoles succinate and 1.5 mg. of NTZ in 1.9 ml. were incubated 15 minutes at 30°. The reaction was stopped with TCA; reduced NTZ was extracted with ethyl acetate and measured at 510 mµ.

*Theoretical nuclear succinic-NTZ reductase to be found if the nuclear NADH-NTZ reductase was mitochondrial in origin.

	Absorbancy at 510 mµ	
Enzyme	NADH	Succinate
Nuclei	0.53	0 (0.065)*
Mitochondria	1.75	0.21

In experiments run coincident with the above, nuclei having no demonstrable succinoxidase were also found to catalyze oxygen uptake with NADH. Table II presents the results of a typical experiment wherein nuclei and mitochondria were isolated from the same homogenate. As with NTZ, nuclei oxidize NADH but not succinate, and mitochondria oxidize both substrates.

Table II

Oxidative Capabilities of Liver Nuclei and Mitochondria

Conditions: 9.3 mg. nuclear protein or 0.7 mg. mitochondrial protein; 450 µmoles sucrose; 180 µmoles Tris, pH 6.9; 36 µmoles $MgCl_2$; and 0.2 µmole cytochrome c, 1.1 µmole NADH and 100 µg. unfractionated liver nuclear histone where indicated; in 5.2 ml. Incubated at 30°C.

Enzyme	Substrate	Addition	µatoms O/hour/mg. protein	
			cytochrome c	
			-	+
Nuclei	NADH	———	84	305
	NADH	Histone		296
	Succinate	———	0	0
Mitochondria	NADH	———	6100	17100
	NADH	Histone	2885	
	Succinate	———	2810	6540

The NADH oxidases of nuclei and mitochondria differ in their susceptibility to histone inhibition. Nuclear NADH oxidase is unaffected by histone; but the oxidase of mitochondria is inhibited by 53%, either

with or without added cytochrome c in the system. Other experiments show that nuclear NADH oxidase has optima at pH 6.9 and 35°C and is inhibited 100% and 67% by 0.2 mM NaCN and 0.5 mM chlorpromazine, respectively. Activity is also eliminated by DNAase treatment, but it may be restored by addition of polyanions such as polyethylene sulfonate or RNA to the system.

By all possible criteria the NADH oxidase is clearly a nuclear activity. Further proof is provided by electron microscopy and low temperature spectroscopy. Examination of the nuclei of Table II at 1200 to 60000 magnification showed one suspect mitochondrion in over 1000 nuclei. In addition, collaborative experiments with Dr. W. B. Elliott (1964) using ultra-sensitive low temperature spectrophotometry have shown six active nuclei preparations to contain only trace amounts of cytochromes b, c and c_1, and no b_5 or a + a_3 (Penniall et al., 1964b).

The important point of Table II is that the nuclei of this experiment require exogenous cytochrome c for maximal activity. In all, some fifteen nuclei preparations have shown activity in NADH oxidation. All of them tested for it have shown no measurable capacity for succinate oxidation by either assay; many preparations show no or only a minimal capacity for NADH oxidase in the absence of exogenous cytochrome c. The requirement by nuclei for exogenous cytochrome c in the presence of an endogenous complement of the enzyme seems anomalous at first consideration. However, nuclei do bind cytochrome c (Beinert, 1951). It is possible therefore that some or all of the endogenous cytochrome c of nuclei is bound in nonfunctional state.

The results of Table III indicate that mitochondria can supply the nuclear requirement for exogenous cytochrome c for NADH oxidation. The rate of NADH oxidation by nuclei plus mitochondria in the absence of cytochrome c is greater than the sum of the rates of each alone, indicating the capacity for interaction reported by Johnson and Ackermann (1953). Separate experiments indicate that NADH is not rate limiting in

these circumstances and that, when heat denatured at pH 7.0, neither nuclei nor mitochondria retain their capacity for this type of interaction. While these experiments do not delineate the predominant path of electron flow in the mixed system, they show that one cannot obtain a true rate for electron transport by nuclei in the presence of mitochondria. In addition, the effects observed for cytochrome c on nuclear activity provide the first indication of a possible means whereby nuclei and mitochondria can interact.

Table III

Cooperative Action of Nuclei and Mitochondria in NADH Oxidation

Conditions: 9.7 mg. nuclear protein and/or 2.2 mg. mitochondrial protein; 1.1 μmole NADH; 0.2 μmole cytochrome c; incubated as indicated in Table II.

Enzyme	μatoms ΔO/hour cytochrome c	
	-	+
Nuclei	1794	5075
Mitochondria	2256	
Nuclei + Mitochondria	7780	

These results verify previous reports that nuclei contain NADH dehydrogenase and cytochrome oxidase, under unequivocal conditions wherein no corrections for mitochondrial activity have been applied. The nature of the nuclear NADH oxidase and its importance to nuclear function are unknown. We feel the key to its understanding lies in sorting out the pertinent relationships between: the stimulation of nuclear activity by exogenous cytochrome c; the capacity of nuclei (Johnson and Ackermann, 1953), or nucleoli (Moses, 1964) to interact with mitochondria; and the familiar external pathway of electron transport of mitochondria (Borst, 1961).

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