Oxidative phosphorylation in liver mitochondria prepared from adrenal-demedullated and epinephrine-treated rats*

The influence of various hormones upon the efficiency of oxidative phosphorylation has been extensively investigated in recent years. However, studies relating to the effect of the adrenal medullary secretions upon these reactions have not appeared except for the brief report of PARK *et al.*[1]. These investigators observed that addition of epinephrine to suspensions of hamster-liver mitochondria depressed somewhat the P/O ratio obtained with β-hydroxybutyrate as substrate. Substrate levels of epinephrine were employed and since its oxidation yields a P/O ratio of 1 in the cytochrome *c* region[2], the lowered P/O ratio may have been due to electron transport from epinephrine and not to its hormonal effects. Because of this apparent interference in studying the effect of epinephrine addition *in vitro*, a different approach was used to analyze the effects of this hormone.

In one series of experiments Wistar rats were injected with 60 μg L-epinephrine/100 g body weight, or with an equivalent volume of normal saline, 30–45 min before sacrificing. In another series, the adrenal medulla was surgically removed three or more weeks before sacrificing the animal. At the time of sacrifice of the demedullated rats, the adrenals were fixed in Bouin's solution and kindly examined histologically by Drs. J. FROMMER and C. MONROE. No medullary tissue was noted and regeneration of cortical tissue appeared complete.

Mitochondria were prepared essentially according to the method of SCHNEIDER AND HOGEBOOM[3]. Mitochondrial suspensions of control and experimental animals were adjusted to approximately equal concentrations on the basis of absorbancy readings at 510 mμ. Succinate oxidation was measured by the conventional Warburg manometric technique and phosphorylation was determined according to the isotope-distribution method of LINDBERG AND ERNSTER[4]. Statistical evaluation of the data involved a paired comparison method[5].

In the aging studies mitochondrial suspensions were incubated in the absence of substrate and Mg^{++} for 0 to 25 min, a condition known to stimulate physical alteration of the mitochondrial structure[6], and subsequently incubated with succinate for 20 min in order to measure their phosphorylative capacity. The data are expressed as the "aging index^p" which is defined as the time of aging, in min, required to depress the phosphorylative capacity of the mitochondria to a value which is half way between the maximal and minimal phosphorylation.

The results of the P/O and aging studies are reported in Table I. Although P/O ratios obtained with mitochondrial preparations of epinephrine-treated rats do not differ from their controls, mitochondria from the former are considerably more resistant to the uncoupling effects of aging. This resistance is greater than would be inferred from the data since in many experiments uncoupling of these preparations had not occurred at the termination of the experiment. In experiments in which loss of phosphorylation had not occurred within the 25-min aging period, the "aging index^p" was taken as 25. This practice would result in mean values somewhat lower than actuality. For further information on this tendency, P/O and aging experiments were repeated with mitochondria from adrenal-demedullated rats. The results re-

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TABLE I

MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION IN EPINEPHRINE-TREATED AND DEMEDULLATED RATS

Each incubation vessel contained: mitochondria, about 1/20th rat liver; orthophosphate, 50 μmoles; KCl, 150 μmoles; adenylic acid, 4.3 μmoles; glucose, 60 μmoles; sucrose, 125 μmoles; succinate, 30 μmoles; Mg^{++}, 7.5 μmoles; hexokinase, 330 K.M. units in P/O studies and 660 K.M. units in aging studies. Final volume, 2.0 ml. Gas phase, air. Temperature, 31°. Time of incubation, 15 or 20 min.

Type of study	*Rats injected with*		*P*	*Conditions of rats*		*P*
	Saline	*Epinephrine*		*Sham-operated*	*Adrenal demedullated*	
P/O	1.59	1.63	> 0.56	1.73	1.69	0.43
Aging index^P	16.1	> 22.6	0.03	16.8	10.9	0.04

corded in Table I reveal no difference in the P/O ratios of mitochondria from sham-operated and demedullated rats, results agreeing with those obtained with epinephrine-treated animals. On the other hand, the "aging index^P" of mitochondria from demedullated rats is lower than that of intact ones, indicating that oxidative phosphorylation is uncoupled more readily in preparations from animals lacking adrenal medullary secretions. These experiments strongly suggest that the adrenal medulla does not promote uncoupling of oxidative phosphorylation; conversely it appears to increase mitochondrial resistance to the uncoupling effects of aging. The physiological significance of this finding requires further investigation.

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