# THE RESPIRATORY CHAIN AND THE OXIDATIVE PHOSPHORYLATION OF RAT BRAIN MITOCHONDRIA

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Received December 15, 1960

Oxidative phosphorylation of rat brain mitochondria was studied in a mannitol-sucrose medium. The medium contained mannitol (0.21 M), sucrose (0.075 M), Tris (tris-hydroxymethyl-amino methane) (0.01 M), and EDTA (ethylenediaminetetraacetate, disodium salt) (0.1 mM) with final pH of 7.4. A similar medium was used previously by Hagihara (1) for the preparation of liver mitochondria and heart sarcosomes. Rat brain was homogenized with 5 volumes of medium in a Potter-Elvehjem homogenizer and the suspension centrifuged at 1,000 g for 10 minutes. The supernatant solution was then centrifuged for 10 minutes at 10,000 g. The sediment was then washed twice with the same medium. The final suspension was obtained from the last sediment by the addition of 0.5 ml of the original medium for each brain used.

The respiratory chain of the rat brain mitochondria was studied by differential recording spectrophotometry at room temperature and at liquid $N_2$ temperature. In a succinate reduced minus oxidized preparation at room temperature the spectrum was used to calculate the concentration of the cytochromes and their relative ratios. For the latter, the concentration of cytochrome a was taken as 1.0. The $\Delta$OD values were estimated at 443, 602, 562 and 550 m$\mu$ for cytochromes $a_3$, a, b, and c + $c_1$, respectively. The $\mu$M concentrations found were 1.2, 0.93, 0.55 and 1.2, respectively with relative ratios of 1.3 (cyt. $a_3$), 1.0 (cyt. a), 0.6 (cyt. b) and 1.3 (cyt. c + $c_1$). The concentration values were calculated from the extinction coefficients reported by Chance (2).

The b and c components were studied in a succinate-antymicin treated preparation. The low temperature spectra displayed two distinct

peaks at 562 and 559 mμ for the b component. The cytochrome $c_1$ peak was at 554 mμ and the cytochrome c peak at 548 mμ.

The oxidative phosphorylation assays were carried out by the method of Chance and Williams (3). The polarographic determinations were made with an oxygen electrode adapted with rotating platinum and calomel reference electrodes in a closed system with a KCl bridge. The oxygen electrode used in this research was built according to an original model of Dr. B. Hagihara to whom the authors are indebted.

Fig. 1 shows the results of the oxidative phosphorylation of the rat brain mitochondria. Glutamate, α-ketoglutarate and succinate were the substrates used.

The experimental sequence and the calculations in the experiments shown in Figure 1, were as follows from left to right. To the air saturated medium (248 μM $O_2$) containing 1.8 ml of a solution of mannitol 0.3M, Tris 0.01 M, EDTA 0.2 mM, KCl 0.01 M and inorganic phosphate 0.01M, 0.2 ml of brain mitochondrial suspension was added; the endogenous respiration was recorded for about 90 sec. at which time 5 mM substrate was added. On subsequent addition of 125 μM ADP, there was an accelerated phase of respiration ('active state') followed by a decrease in respiration after the ADP became depleted. The P/O ratios were calculated as ADP/O from the uptake of oxygen in μ atoms per liter during the active state of respiration and the molar concentration of ADP added. The respiratory control coefficient was calculated as the ratio of the respiratory rates (in μM $O_2 . sec^{-1} . liter^{-1}$) with ADP and the control respiration after ADP was consumed. The ADP/O ratios of rat brain mitochondria were of the order of 1.88 for glutamate, 1.98 for succinate and 2.84 for α-ketoglutarate. The ADP/O ratios for glutamate and for succinate were identical in all the assays carried out.

The preparations showed good respiratory control. However, in the presence of magnesium or in the absence of versene, the mitochondria failed to show respiratory control, even though magnesium increased the rate of α-glycerophosphate oxidation as previously found by Sacktor et al. (4). It was also found that glutamate respiration is stimulated by 2,4-dinitrophenol in the presence of inorganic phosphate;

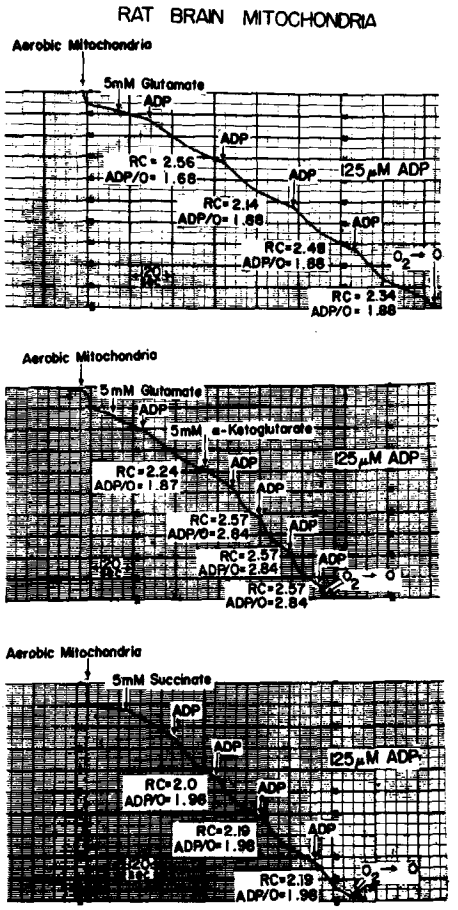


Fig. 1. Polarographic assay of respiration and oxidative phosphorylation of rat brain mitochondria. The figure shows three different experiments with glutamate (top), glutamate and succinate (middle) and succinate (bottom) as substrates. Respiratory control, RC, is calculated as the ratio of respiration rates with and without ADP.

under these conditions the rate of respiration is almost two times greater than that in the absence of inorganic phosphate.

The authors wish to thank Prof. Britton Chance, Johnson Foundation, University of Pennsylvania, for his discussion of several topics of this work, and also, for the using of special equipment and facilities in his laboratory.

## References

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