

# EFFECT OF SUBSTRATE ON RESPIRATION COUPLED WITH PHOSPHORYLATION IN THE LUMBAR DIVISION OF THE SPINAL CORD

A. I. Dvoretiskii and A. D. Reva

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The intensity of oxidation and phosphorylation in homogenates of the lumbar division of the cat spinal cord varies with the oxidation substrate used.

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It has been shown that the intensity of oxidative phosphorylation in mitochondrial preparations and brain homogenates is largely dependent on the nature of the oxidation substrate [1, 10, 11, 13]. These results have been obtained chiefly by investigation of the brain. No concrete data for the spinal cord on this problem are available.

As part of a series of investigations into the physiology and biochemistry of the spinal cord [3-8], a study was made of phosphorylation coupled with biological oxidation of various substrates (glucose, pyruvate, citrate,  $\alpha$ -ketoglutarate, succinate, fumarate, malate, glutamate,  $\gamma$ -aminobutyrate, and NAD.H) in the tissues of the lumbar division of the cat spinal cord.

## EXPERIMENTAL METHOD

The intensity of oxygen utilization by the spinal cord tissues was determined manometrically by Warburg's method [2]. Incubation was carried out in the medium of Dahl and Samson [14] with slight modifications. The intensity of oxidative phosphorylation was determined by the decrease in inorganic phosphorus in the samples, determined by Lowry's method [16] as modified by Skulachev [9].

## EXPERIMENTAL RESULTS

The results of investigations of phosphorylation and their coupling with oxidation of the substrates mentioned above in tissues of the lumbar enlargement of the cat spinal cord are given in Table 1.

The results given in Table 1 show that the intensity of respiration and phosphorylation and of their coupling in the tissues of the lumbar division of the spinal cord vary depending on which oxidation sub-

TABLE 1. Phosphorylation Coupled with Biological Oxidation of Various Substrates in Lumbar Division of Spinal Cord

Substrate	No. of expts.	No. of ani- mals	$\Delta O$ in $\mu atom$	$\Delta P$ in. $\mu atom$	P/O
Control (without substrate)	6	6	$0.35 \pm 0.02$	$0.56 \pm 0.04$	$1.57 \pm 0.12$
Glucose	6	6	$0.35 \pm 0.02$	$0.55 \pm 0.03$	$1.57 \pm 0.10$
Pyruvate	6	6	$0.54 \pm 0.02$	$1.06 \pm 0.14$	$1.92 \pm 0.06$
Citrate	6	6	$0.33 \pm 0.03$	$0.35 \pm 0.03$	$1.04 \pm 0.08$
$\alpha$ -ketoglutarate	8	15	$0.45 \pm 0.03$	$0.85 \pm 0.04$	$2.03 \pm 0.12$
Succinate	11	16	$0.59 \pm 0.03$	$0.91 \pm 0.04$	$1.55 \pm 0.06$
Fumarate	6	6	$0.45 \pm 0.02$	$0.72 \pm 0.03$	$1.63 \pm 0.09$
Malate	6	6	$0.41 \pm 0.03$	$0.68 \pm 0.04$	$1.64 \pm 0.07$
Glutamate	10	20	$0.49 \pm 0.03$	$0.99 \pm 0.07$	$2.05 \pm 0.12$
$\gamma$ -aminobutyrate	6	6	$0.34 \pm 0.03$	$0.61 \pm 0.03$	$1.64 \pm 0.14$
NAD.H	6	6	$0.45 \pm 0.04$	$0.60 \pm 0.02$	$1.37 \pm 0.09$

Note: O represents oxygen utilized by 10 mg tissue per hour; P consumption of inorganic phosphorus by 10 mg tissue per hour; P/O represents ratio for various oxidation substrates.

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strate is used. Oxidation and phosphorylation took place at the highest intensity when succinate, pyruvate, and glutamate were used as oxidation substrates, and at the least intensity when citrate, GABA, and glucose were used.  $\alpha$ -Ketoglutarate, NAD.H, and malate occupied an intermediate position for intensity of oxidation and phosphorylation. The coefficient of oxidative phosphorylation (P/O) was highest for oxidation of  $\alpha$ -ketoglutarate, glutamate, and pyruvate, somewhat lower for oxidation of malate, fumarate,  $\gamma$ -aminobutyrate, glucose, and succinate and lowest for oxidation of NAD.H and citrate.

The low level of oxidation and phosphorylation associated with the utilization of endogenous substrates was determined by their low concentration in the cells. This hypothesis is confirmed by the fact that substrates of the tricarboxylic cycle never accumulate in large quantities in nerve cells during their activity [17]. Meanwhile, nerve tissue always contains large quantities of glutamate, aspartate, and glutamine [12]. During respiration at the expense of endogenous substrates, oxygen utilization is associated mainly with the oxidative breakdown of glutamate [18].

Involvement of oxidation substrates in other phases of metabolism unconnected with oxidative phosphorylation [15] and the degree of permeability of the mitochondria for these substrates may possibly influence the levels of oxidation and phosphorylation.

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