The respiratory activity and respiratory control of sarcosomes isolated from the thoracic muscle of the housefly

Although it is known that the thoracic muscles of flying insects are rich in cytochrome[1] and in large mitochondria (sarcosomes)[2,3], and have *in vivo* a high respiratory activity which is controlled by the muscular activity[4], it has proved difficult to isolate sarcosomes with the properties to be expected from these findings. Oxidative phosphorylation was not demonstrated until 1953 (ref. 5, 6), while respiratory control (*i.e.* dependence of the rate of respiration on the concentration of ADP) has only very recently been achieved[7,8]. The values for the P:O ratio and the Q_{O_2} (μl O_2/mg sarcosomal protein/h) which have been reported are much lower than those expected from the activity *in vivo*[9–11].

In the present report it is shown that, if sarcosomes isolated from the thoracic muscle of the housefly *Musca domestica*, essentially by the method of LEWIS AND SLATER[5], are tested in the presence of a supernatant fraction, high Q_{O_2}'s, P:O ratios and respiratory control can be measured. A typical experiment with succinate as substrate is shown in Fig. 1 and average values are given in Table I. The addition of the supernatant fraction caused a large increase in the respiratory rate and in the P:O ratio, with the establishment of respiratory control. In other experiments, Q_{O_2}'s of 695*, 486, and 458 were obtained under similar conditions with α-glycerol phosphate (0.05 *M*), DPNH (0.05 *M*) and pyruvate (0.02 *M*) + malate (0.002 *M*), respectively, as substrate.

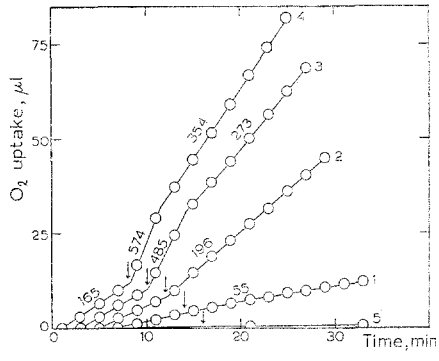

Fig. 1. The effect of supernatant fraction on the respiration of sarcosomes isolated from housefly thoracic muscle. 200 thoraces from flies 8–18 days old were gently pounded in a mortar with 5 ml 0.25 *M* sucrose, 1 m*M* EDTA, pH 7.4, and the brei filtered through muslin. The filtrate was centrifuged at 150 × *g* for 3 min and the supernatant centrifuged for 8 min at 3000 × *g*. The sedimented sarcosomes were suspended in 4 ml isolation medium. The supernatant fraction contained 3.34 mg protein/ml. The reaction mixture contained 15 m*M* KCl, 2 m*M* EDTA, 5 m*M* $MgCl_2$, 0.05 *M* Tris-acetate buffer, 0.03 *M* potassium phosphate, 0.1 m*M* ATP, 0.06 *M* succinate, 0.63 mg sarcosomal protein. The pH was 7.5, the reaction volume 1 ml and the temp. 25°. At the arrow, 3 μmoles ADP were added from the side bulb. Curve 1, no further addition; curves 2, 3 and 4, with 0.1, 0.2 and 0.3 ml supernatant fraction, respectively; curve 5, with 0.3 ml supernatant fraction without sarcosomes. The values given over straight parts of the curves are Q_{O_2}'s (μl O_2/mg sarcosomal protein/h).

Similar results were obtained by working with the suspension obtained by filtering the muscle brei through muslin. The mean P:O ratio (number of experiments in brackets) of these homogenates were: succinate, 2.03 (10); α-ketoglutarate (in the presence of 0.02 *M* malonate), 2.27 (3); glutamate, 2.30 (3). The P:O ratios were

Abbreviations: ADP, ATP, adenosine di- and triphosphate; EDTA, ethylenediaminetetraacetic acid; DPNH, reduced diphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.

* This is the average rate over 30 min. The initial Q_{O_2} was 783.

unaffected by varying the concentration of added adenine nucleotide between zero and 1.8 m*M* (contrast ref. 12). In the presence of ADP, the rate of respiration was increased 11-fold by adding inorganic phosphate, with either succinate or α-ketoglutarate as substrate.

TABLE I

RESPIRATORY ACTIVITY, RESPIRATORY CONTROL AND OXIDATIVE PHOSPHORYLATION WITH SARCOSOMES ISOLATED FROM HOUSEFLY THORACIC MUSCLE, WITH SUCCINATE AS SUBSTRATE

The values given are means with the number of experiments in brackets.

Addition	*Respiratory activity* μl O_2/mg sarcosomal protein/h*	*Respiratory-control index***	*P:O**
None	58 (7)	1.0 (3)	0.59 (3)
Serum albumin (2 %)	49 (4)	1.0 (1)	1.21 (2)
0.1 ml supernatant fraction	270 (2)	1.87 (3)	1.88 (2)
0.2 ml supernatant fraction	538 (2)	3.83 (3)	2.20 (2)
0.3 ml supernatant fraction	625 (3)	3.51 (5)	2.25 (2)

* Measured as in Fig. 1, but with 0.03 *M* glucose + hexokinase in place of ADP. P:O ratios determined by method of LEWIS AND SLATER[5].

** (Q_{O_2} in presence of ADP): (Q_{O_2} in presence of 0.1 m*M* ATP).

Further studies are required to elucidate the mechanism of action of the supernatant fraction. Its effect is abolished by heating for 5 min at 100°. In any case, it is clear that the isolated sarcosomes are capable of a high activity in the Krebs cycle, and that this oxidation is accompanied by oxidative phosphorylation and is under control of the ADP concentration. The respiratory rates measured in the presence of ADP, which are much higher than those previously reported, approach those found *in vivo*[4,9–11].

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