## Influence of phosphorylcholine on endogenous oxidative phosphorylation of rat-liver mitochondria

As previously demonstrated[1], phosphorylcholine causes, both *in vivo* and *in vitro*, reassociation of oxidative phosphorylation in partly uncoupled fatty-liver mitochondria, when pyruvate is used as substrate. This was tentatively attributed to the inhibition by phosphorylcholine of endogenous fatty acid oxidation. It is evident that such an inhibition would give rise to an increase in the P:O ratio because endogenous fatty acid oxidation requires energy ( ~ P), which is available even when hexokinase and glucose are present outside the mitochondrial membrane, in contrast to pyruvate oxidation which does not need this supply of energy. Since rat-liver mitochondria contain an appreciable amount of endogenous substrates—probably free fatty acids[2]—capable of sustaining a remarkable oxidative phosphorylation[3-6], it seemed of interest to compare the action of phosphorylcholine on the "endogenous" oxidative phosphorylation in normal and in fatty-liver mitochondria.

The oxidation of endogenous substrates was followed by measuring the oxygen uptake in the Warburg apparatus and the esterification of inorganic phosphate by determining glucose 6-phosphate according to STEINER AND WILLIAMS[7]. Correction for glucose 6-phosphate synthesised by myokinase and hexokinase has been made by measuring, according to SLATER[8], glucose 6-phosphate formed in the presence of KCN (0.01 *M*). The reaction mixture consisted of: 0.3 ml of 0.1 *M* $KH_2PO_4$; 0.4 ml of 0.03 *M* $MgCl_2$; 0.3 ml of 0.5 *M* sucrose; 0.3 ml of 0.2 *M* glucose; 0.1 ml of 0.4 *M* KF; 4 $\mu$moles ADP and 8 $\mu$moles AMP; hexokinase powder (Sigma, practical type III) 800 $\mu$g. In some experiments 3 $\mu$moles of phosphorylcholine were added. The final volume was of 2.5 ml and pH 7.4. Rat-liver mitochondria were prepared according to SCHNEIDER AND HOGEBOOM[9] in 0.25 *M* sucrose and added to the reaction mixture in amounts corresponding to 2 mg nitrogen, as determined by JOHNSON's method[10]. Fatty liver was induced by feeding the animals a low-protein, high-fat diet[1].

The results reported in Fig. 1 and in Table I indicate that, in fatty-liver mitochondria, both phosphorylation and $O_2$ uptake are considerably reduced and the P:O ratio is significantly decreased. Both in normal and fatty-liver mitochondria

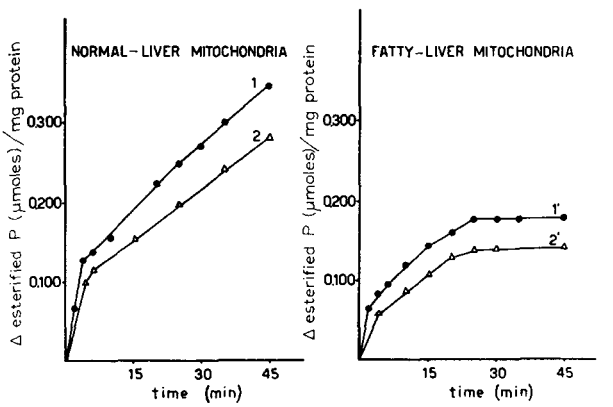


Fig. 1. Phosphorylation coupled to oxidation of endogenous substrate. Curves 1 and 1', without addition of phosphorylcholine; curves 2 and 2', with addition of phosphorylcholine. Each curve is the average of 6 experiments.

TABLE I

Effect of phosphorylcholine on the P:O ratio of normal and fatty-liver mitochondria without addition of any substrate. The data were obtained in the interval between the 20th and the 45th min of incubation. Average values referred to 1 mg protein.

| *Group* | *Number of experiments* | *Phosphorylcholine added (μmoles)* | *$O_2$ uptake (μatoms)* | *Esterified P (μmoles)* | *P:O* |
|---|---|---|---|---|---|
| Normal | 6 | — | 0.200 ± 0.019* | 0.120 ± 0.012 | 0.60 ± 0.10 |
| Normal | 6 | 3 | 0.098 ± 0.009 | 0.083 ± 0.010 | 0.84 ± 0.13 |
| Fed high-fat diet | 6 | — | 0.079 ± 0.010 | 0.028 ± 0.005 | 0.35 ± 0.07 |
| Fed high-fat diet | 6 | 3 | 0.026 ± 0.006 | 0.015 ± 0.007 | 0.57 ± 0.05 |

* Standard deviation.

phosphorylcholine reduces P esterification and to a greater extent $O_2$ uptake, so that the P:O ratio is significantly increased.

The observed decrease of respiration in fatty-liver mitochondria could be attributed, on the basis of our previous experiments[1], to a decrease of endogenous fatty-acid oxidation, consequent to an impairment of the reactions leading to acetoacetate formation. Subsequent accumulation of free fatty acids which, according to HÜLSMANN *et al.*[11] and LEHNINGER AND REMMERT[12] would uncouple oxidative phosphorylation, might be responsable for the observed decrease of the P:O ratio. Alternatively, the present results can be attributed to an impaired synthesis of phospholipids[13–15].

Phosphorylcholine increases the P:O ratio both in normal and fatty-liver mitochondria. This action is consistent with the previously proposed hypothesis that phosphorylcholine provides a building block for the synthetic removal of uncoupling free fatty acids from the mitochondria by converting them into phospholipids.

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C. R. ROSSI
L. SARTORELLI
N. SILIPRANDI

*Institute of Biochemistry,*
*University of Padova, Padova (Italy)*

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