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## SHORT COMMUNICATION

# Respiratory Control Index of Mitochondria Isolated from Regenerating Rat Liver

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## Summary

**Mitochondria were isolated from regenerating rat liver 12, 24 and 48 h after partial hepatectomy. The "State 3" and "State 4" respiration were measured in the presence of succinate. The P/O quotient and respiratory control index (RCI) were calculated. The experimental data showed that the partial uncoupling of oxidative phosphorylation in regenerating liver mitochondria occurring in the early period of regeneration is partly due to free fatty acids.**

**Key words**

Rat liver – Regeneration – Mitochondria – Respiratory control index (RCI) – P/O quotient

Normal hepatocytes do not proliferate actively when the postnatal growth of the liver is terminated. A proliferation process can, however, be induced by hepatic injury caused by various chemical agents (Červinková *et al.* 1987) or by partial hepatectomy (Bucher 1963, Červinková *et al.* 1985). Recovery of the tissue mass in the regenerating liver is dependent on the supply of energy. There are indications that early changes in cellular energy metabolism are one of the possible events that initially trigger liver regeneration (Campbell *et al.* 1990, Skullman *et al.* 1991).

It was found that, in the early period of liver regeneration, the respiratory control index (RCI) is depressed in parallel to the decrease of mitochondrial ATPase activity (Buckle *et al.* 1985, 1986, Guerrieri *et al.* 1994). This means that both capacity and efficiency of ATP production system are depressed. The decrease of ATPase activity is due to a lower content of the $F_1$ subunit of the enzyme in the mitochondrial membrane (Guerrieri *et al.* 1994). Nevertheless, the mechanism of the lower effectiveness of the energy transformation system is not quite clear. The efficiency of the energy transformation process is dependent on the ability of the inner mitochondrial membrane to maintain the proton electrochemical gradient. A proton leak decreasing the efficiency of energy transformation may occur as a physiological process through a regulated proton channel or as a pathological process due to impairment of the inner mitochondrial membrane by various endogenous or exogenous protonophores or detergents (Garlid *et al.* 1989).

In previous experiments, the decrease of respiratory control index was explained by a lower rate of "State 3" respiration (Buckle *et al.* 1985, Guerrieri *et al.* 1994). This is not in complete agreement with the fact that uncoupler-induced respiration was not depressed and with the other findings that decreased rate of succinate oxidation was not observed (Gear 1970, Nagino *et al.* 1989, Bláha *et al.* 1994). The question therefore arises to what extent other factors inducing the proton leak of the mitochondrial membrane may participate. Mitochondria can be

partially uncoupled by various endogenous substances present in the cytosol which influence the energization state of mitochondria and decrease the efficiency of the energy transformation process. Free fatty acids, known as uncoupling agents, could be responsible for this effect. It is well known that a high increase of serum free fatty acids occurs during the first days of liver regeneration (Schoffield *et al.* 1985, 1987). We have therefore tested in our experiments to what extent bovine serum albumin may eliminate the decrease of respiratory control index of mitochondria isolated from the regenerating liver. This should indicate the possible participation of free fatty acids in modifying the energization state of mitochondria.

The experimental animals were 3-month-old male Wistar rats fed a standard laboratory diet. Partial (70 %) hepatectomy was performed as described by Higgins and Anderson (1931). Mitochondria were isolated according to Schneider and Hogeboom (1950). Mitochondrial oxygen consumption was measured with a Clark oxygen electrode. Mitochondria were incubated at 30 °C in 1 ml of the medium containing 100 mM KCl, 10 mM Tris-HCl, 0.5 mM EDTA, 2 mM $MgCl_2$, 4 mM K-$PO_4$, pH 7.4. Succinate was 10 mM. Where indicated, 200 n-moles ADP and 5 mg of bovine serum albumin (fatty acid poor) were added. Mitochondrial protein in 1 ml of the incubation mixture was 0.3–0.6 mg.

The measured values were expressed as means ± S.E.M.. Statistical differences between control and treated animals were evaluated by Student's t-test. The values were considered to be significantly different when $p<0.05$.

**Table 1**
Respiration of mitochondria isolated from regenerating liver

| Hours after partial hepatectomy | n-atoms O / min / mg mitochondrial protein "State 4" (−ADP) −BSA (A) | +BSA (B) | (B/A) | "State 3" (+ADP) −BSA (A) | +BSA (B) | (B/A) |
|---|---|---|---|---|---|---|
| 0 | 11.3±0.5 (100 %) | 10.2±1.7 (100 %) | 0.90 | 58.9±7.3 (100 %) | 68.6±3.4 (100 %) | 1.16 |
| 12 | 16.8±1.0 (148 %)* | 12.2±1.1 (120 %)* | 0.73 | 61.8±2.5 (105 %) | 75.6±9.2 (110 %) | 1.22 |
| 24 | 26.5±0.5 (234 %)** | 19.7±2.5 (193 %)** | 0.74 | 58.9±5.1 (100 %) | 74.6±9.3 (109 %) | 1.27 |
| 48 | 27.5±1.1 (243 %)** | 19.8±1.5 (194 %)** | 0.72 | 77.0±5.2 (131 %)* | 79.8±5.1 (116 %) | 1.03 |

*Data are means ± S.E.M. calculated from four animals, * and ** indicate significant increase ($p<0.01$ and $p<0.001$, respectively.*

Table 1 shows that "State 4" respiration in the absence of BSA was markedly activated during the regeneration process under our experimental conditions. "State 3" respiration in the absence of BSA was not depressed. These data thus confirm our previous findings (Guerrieri *et al.* 1994) showing a decrease of RCI, however, the mechanism of this change is different (Tables 1 and 2).

In the early period of regeneration very complicated metabolic processes occur. During the dedifferentiation period many metabolic changes resembling foetal tissue were described as well as transition of hepatocyte hypertrophy to cell hyperplasia during the redifferentiation period (Izquierdo *et al.* 1990, Aloni *et al.* 1992). These processes of dedifferentiation and subsequent redifferentiation and recovery of liver mass include many factors that are not yet fully understood. This could be responsible for the different findings obtained in various laboratories. As far as succinate oxidation by isolated mitochondria from regenerating liver is concerned, a decrease was described by Guerrieri *et al.* (1994), no changes were found in our experiments (Table 1) and by Gear (1970) whereas an increase was reported by Nagino *et al.* 1989).

Our data thus show that about 30 % of the RCI decrease after partial hepatectomy may be eliminated by bovine serum albumin. The residual increase of "State 4" respiration also indicates an increase of inner membrane proton permeability.

In agreement with previous findings (Guerrieri *et al.* 1994) we found no changes of the P/O ratio during the regeneration period. Individual values varied in the range between 1.7–1.9.

**Table 2**
Respiratory control index (RCI) of mitochondria isolated from regenerating liver

| Hours after partial hepatectomy. | RCI −BSA (A) | RCI +BSA (B) | (B/A) |
|---|---|---|---|
| 0 | 5.2±0.5 (100 %) | 6.7±0.8 (100 %) | 1.17 |
| 12 | 3.7±0.2 (71 %)* | 6.2±1.2 (92 %) | 1.67 |
| 24 | 2.2±0.2 (42 %)** | 3.8±0.4 (57 %)** | 1.72 |
| 48 | 2.8±0.1 (54 %)** | 4.0±0.3 (60 %)** | 1.42 |

*Data are means ± S.E.M. calculated from four animals, * and ** indicate significant differences ($p<0.01$ and $p<0.001$, respectively*]

We may conclude from our data that free fatty acids together with other factors participate in partial uncoupling of mitochondria isolated from the regenerating liver during the early prereplicative phase. To understand completely all these metabolic processes accompanying recovery of liver mass after partial hepatectomy, further experiments will be required. More experimental data are also necessary in order to establish better conditions for protecting the regenerating liver against factors induced by postoperative stress, e.g. the action of free radicals (Koudelová *et al.* 1994, Rauchová *et al.* 1995), and to propose appropriate nutritional formulas supporting the regeneration process.

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