# ACTION OF 'DIAMIDE' ON SOME ENERGY LINKED PROCESSES OF RAT LIVER MITOCHONDRIA

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## 1. Introduction

It has been previously shown that some energy linked processes, partially lost during the ageing of mitochondria, such as respiratory control index, ADP:O ratio, and the $Ca^{2+}$ and $K^{+}$ uptake, are restored by addition of dithioerythritol (DTE) to aged mitochondria [1]. It was consequently suggested that the maintenance of mitochondrial energy linked processes might depend upon the integrity of pairs of vicinal thiol groups in the inner membrane.

To test such a hypothesis the action of 'diamide' on rat liver mitochondria has been studied.

Thiol oxidizing agents, such as 'diamide' (diazinedicarboxylic acid bis dimethylamide) can alter the thiol-disulfide balance and yet not interfere, within certain limits, with return to apparent normality [2].

The results reported in the present paper show that treatment of freshly prepared rat liver mitochondria with 'diamide' brings about, like spontaneous ageing, a loss, or an impairment of some typical energy linked processes, which can be restored by successive addition of DTE to functionally damaged mitochondria.

## 2. Experimental

Rat liver mitochondria were isolated in 0.25 M sucrose following Schneider and Hogeboom [3]. Mitochondrial protein was determined by a biuret method. Oxygen uptake was measured with a Clark oxygen electrode. $Ca^{2+}$ uptake was measured isotopically with $^{45}Ca^{2+}$ as a tracer; after rapid centrifugation on mitochondrial pellets, suspended in Packard 'Instagel', were counted by liquid scintillation. $Ca^{2+}$ movements were followed by recording simultaneously the pH and oxygen uptake with a combination pH electrode and a Clark electrode, respectively. ATPase activity was estimated from the pH records [4].

## 3. Results and discussion

As shown in fig. 1, the respiratory control index with succinate as substrate, greatly decreased upon preincubation of rat liver mitochondria in the presence of 0.1 mM 'diamide' (compare trace A and B). Successive addition of DTE restored respiratory control to approximately normal values (trace C). In the presence of glutamate and malate it was observed that the oxygen uptake by the 'diamide' treated mitochondria progressively slowed down, owing to a leakage of $NAD^{+}$ from mitochondria, produced by the 'diamide' treatment, as described elsewhere [5]. However DTE (compare trace F with trace E) also prevented such a progressive diminution of respiration.

Treatment with 'diamide' also affected the capacity of rat liver mitochondria to take up and to retain $Ca^{2+}$.

Table 1 shows that $^{45}Ca^{2+}$ uptake by rat liver mitochondria was significantly inhibited by 'diamide' and that DTE, successively added, fully restored $^{45}Ca^{2+}$ uptake.

Moreover, the typical experiment reported in fig. 2 shows that in 'diamide' treated mitochondria the suboptimal amount of $Ca^{2+}$ accumulated (monitored in these experiments by measuring the simultaneous $H^{+}$

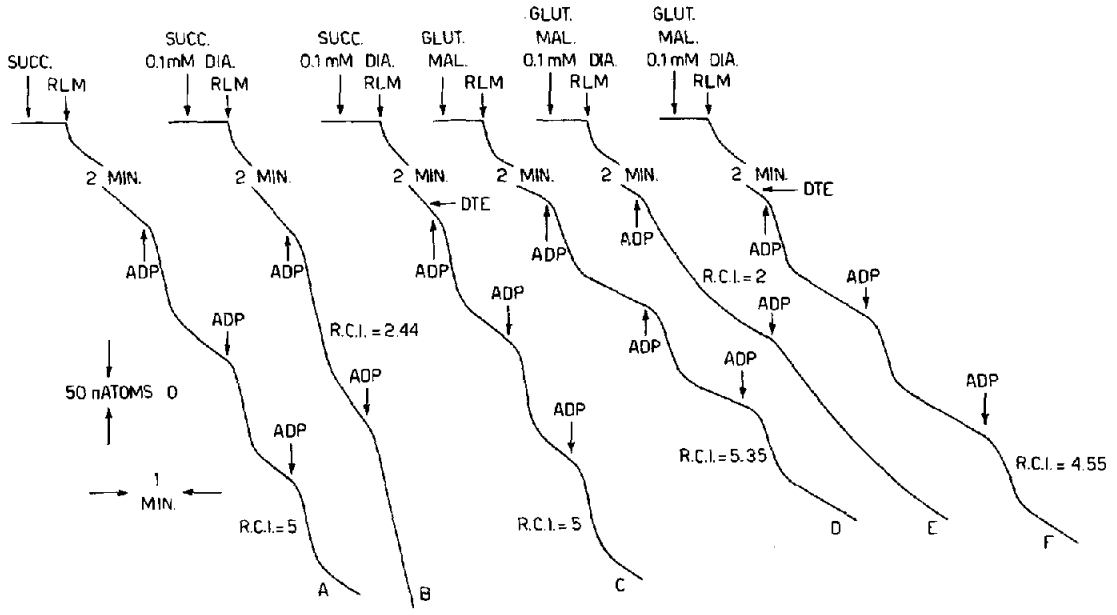


Fig. 1. Effect of 'diamide' and dithioerythritol on the respiratory control of rat liver mitochondria. The assay medium (final volume 1.8 ml temperature 20°C) contained 10.8 mM $K_2HPO_4$, 2.82 mM $KH_2PO_4$, 9.9 mM NaF, 21.62 mM NaCl, 48.3 mM KCl, 5 mM $MgCl_2$. Substrates were 5 mM. Traces A–B–C 5 μM rotenone is present. 5 mg of mitochondrial protein (RLM) were added. Traces C–F: DTE added was 0.25 mM. Where indicated 220 nmoles and 300 nmoles of ADP were added in traces A–B–C and D–E–F, respectively.

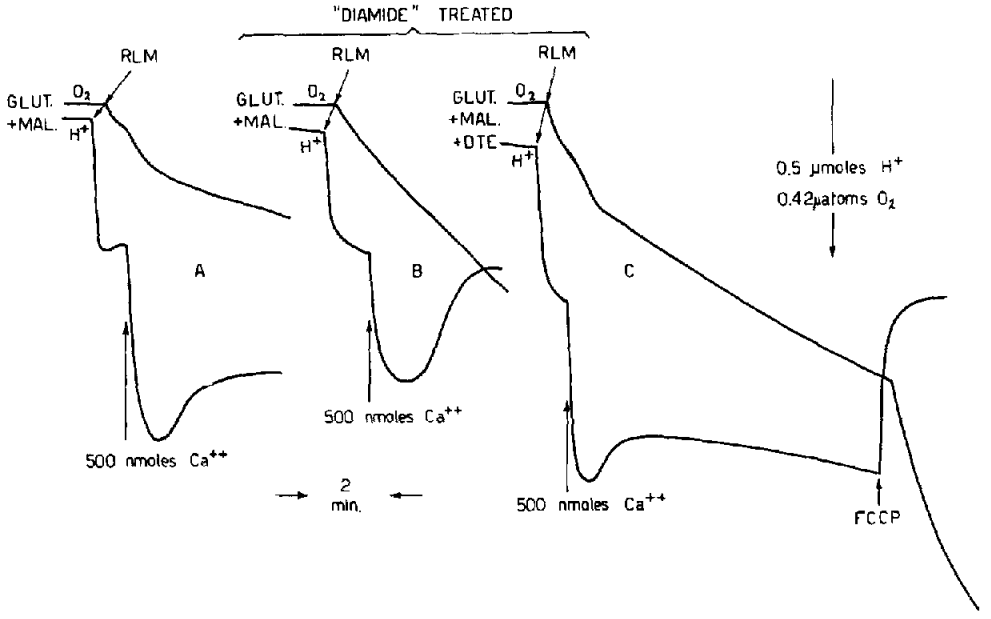


Fig. 2. Effect of 'diamide' and dithioerythritol on $Ca^{2+}$ uptake by rat liver mitochondria. Experimental conditions as in table 1. Trace C: 1 mM DTE is present.

Table 1
Effect of dithioerythritol on $^{45}Ca^{2+}$ uptake by 'diamide' treated mitochondria

| $^{45}Ca^{2+}$ added cpm | $^{45}Ca^{2+}$ uptake cpm | | |
|---|---|---|---|
| | Control | 'Diamide' - treated | |
| | | – DTE | +DTE |
| 3475 | 3172 | 219 | 2869 |
| 3525 | 2744 | 235 | 2815 |
| 2650 | 1983 | 172 | 1913 |

The mitochondria (50 mg/ml 0.25 M sucrose) have been preincubated at 4°C for 6 min. in the presence of 'diamide' (60 nmoles/mg protein). The assay medium (final volume 4 ml, temperature 20°C) contained 80 mM KCl, 5 mM Tris–HCl pH 7.4, 5 mM glutamate, 5 mM malate. Where present DTE was 1 mM. 10 mg of mitochondrial protein was used for each assay; after 1 min incubation 500 nmoles of $Ca^{2+}$ (labelled with $^{45}Ca^{2+}$) were added and after 2 min incubation the mitochondrial pellet was counted.

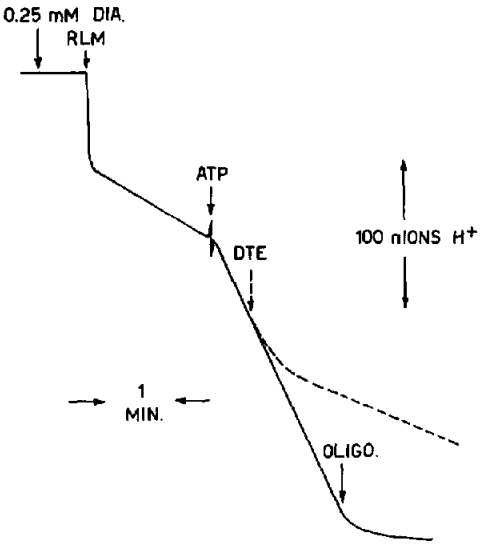


Fig. 3. Effect of 'diamide' and dithioerythritol on ATPase activity of rat liver mitochondria. The assay medium (final volume 2 ml, temperature 20°C) contained 80 mM KCl, 5 mM Tris–HCl pH 7.4, 0.25 mM 'diamide'. 5 mg of mitochondrial protein (RLM) were added. Additions: 0.5 mM ATP, 1 mM DTE, 5 μg oligomycin.

ejection) was not retained within mitochondria during the resting respiration, but spontaneously released into the external medium (compare trace A and B). In the presence of DTE, $Ca^{2+}$ were taken up and retained in 'diamide' treated mitochondria until the energy coupling mechanism was interrupted by FCCP (see Trace C).

It was finally demonstrated that 'diamide' induced a stimulation of ATPase activity of rat liver mitochondria, (as indicated by $H^+$ release) which was inhibited by a successive addition of DTE (fig. 3).

Considering the mechanism of 'diamide' action [2], the reported results can be referable to an oxidation of pairs of thiol groups reversed by DTE. The analogy between the alterations induced by 'diamide' as well as those produced by spontaneous ageing of mitochondria [1], and the fact that in both conditions DTE restored to normality the perturbed processes, indicate that both spontaneous ageing and 'diamide' treatment bring about an oxidation of pairs of vicinal thiols. It is therefore reasonable to assume that a proper SH/SS balance is a critical condition for the maintenance of mitochondrial membrane 'tightness' and for the coupling efficiency of mitochondria.

## References

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