# THE ACTION OF TELLURITE, A REAGENT FOR THIOL GROUPS, ON MITOCHONDRIA OXIDATIVE PROCESSES

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Summary: Tellurite, a reagent for thiol groups, added to rat kidney, or liver mitochondria at the concentration of 1 mM, selectively inhibits the oxidation of NAD dependent substrates (pyruvate, α-ketoglutarate, glutamate, etc.) without affecting the oxidation of succinate, α-glycerophosphate and ascorbate. Moreover NADH oxidation by rat heart or by aged liver mitochondria is not affected by tellurite.
The inhibitory effect of tellurite, which unlike that of rotenone is not reversed by menadione, is completely reversed by dithioerythritol.

The inhibitory action of tellurite on enzymes was investigated many years ago in crude preparations and was attributed to an oxido-reductive interaction of tellurite with SH groups (1-4).

Since then, tellurite has no longer been employed in enzyme investigations, neither at the molecular nor the supramolecular level.

In the present communication some effects of tellurite on mammalian mitochondria are reported. The results indicate that this thiol reagent specifically inhibits the oxidation of NAD dependent substrates in intact mitochondria.

As shown in Table I, at 1 mM concentration, tellurite completely inhibited the oxidation of NAD substrates by rat kidney mitochondria in state 3. The same inhibition occurred

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TABLE I

EFFECT OF 1 mM TELLURITE ON THE OXIDATION OF DIFFERENT SUBSTRATES BY KIDNEY RAT MITOCHONDRIA

| Substrates + tellurite | % Inhibition $O_2$ uptake | P/O |
|---|---|---|
| Pyruvate + oxaloacetate | 100 | - |
| α-ketoglutarate | 100 | - |
| D-L isocitrate | 100 | - |
| D-L β-hydroxybutyrate + + oxaloacetate | 100 | - |
| D-L malate | 100 | - |
| Glutamate | 100 | - |
| Succinate | 0 | 1.69 |
| D-L α-glycerophosphate | 0 | 1.70 |
| Ascorbate + TMPD | 0 | 0.90 |

Kidney rat mitochondria (5 mg protein) prepared by the procedure of Schneider and Hogeboom (5) in 0.25 M Sucrose + + 1 mM EDTA were suspended in a medium containing 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 $MgCl_2$; final volume was 1.8 ml. 1 mM ADP was added. Substrates were 10 mM; oxaloacetate 0.25 mM; TMPD 0.1 mM; tellurite ($K_2TeO_3$) 1 mM.

$O_2$ uptake was measured at 25°C with a Clark oxygen electrode. ATP was determined by the method of Steiner and Williams (6).

also in the uncoupled state in the presence of DNP. At the same concentration tellurite did not affect the oxidation of succinate, α-glycerophosphate and ascorbic acid, nor the phosphorylative efficiency of their oxidation, as it can be deduced from the P:O values.

The same results have been obtained with rat liver mitochondria.

Apparently, tellurite action resembles that of amytal or rotenone. Nevertheless, the results reported in Figs. 1 and 2 clearly show that tellurite did not affect the electron flux through the respiratory chain.

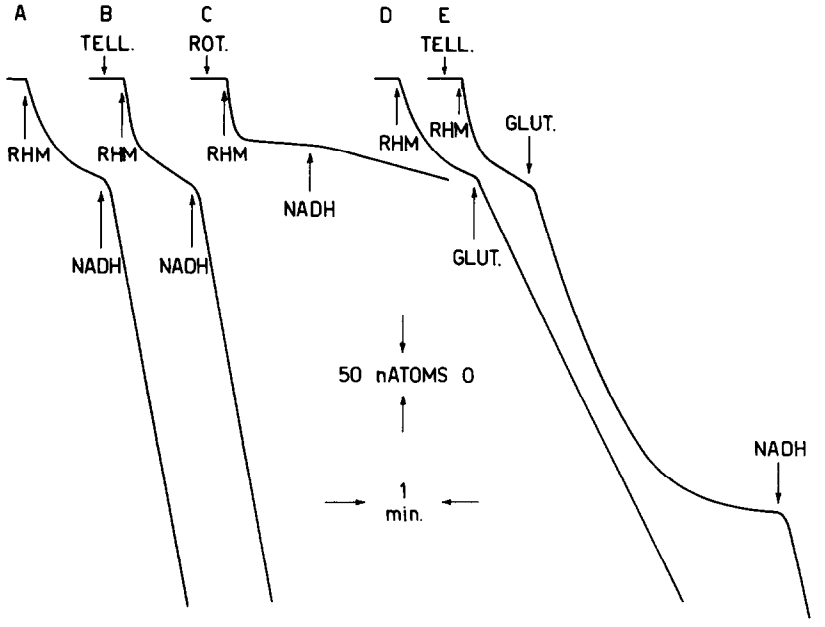


Figure 1: Oxidation of glutamate and NADH by rat heart mitochondria (RHM).

Experimental conditions and incubation medium as in Table I. 1 mM ADP was present.
Additions at the points indicated by arrows: heart mitochondria (3 mg protein) isolated according to Holton et al. (7) in 0.25 M sucrose + 1 mM EDTA, 2 mM NADH, 2 mM $K_2TeO_3$ (tell.), 0.1 mM rotenone, 10 mM glutamate.

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Fig. 1, shows, in fact, that tellurite did not inhibit, unlike rotenone, NADH oxidation by rat heart mitochondria preparation, or (results not shown), by aged liver mitochondria. It can also be observed that at the same concentration tellurite did inhibit the oxidation of glutamate (trace E).

Moreover, Fig. 2 clearly shows that in rat liver mitochondria, the inhibition of α-ketoglutarate oxidation induced by 1 mM tellurite was not reversed by the addition of menadione (trace A), while rotenone inhibition was overcome upon addition of menadione (trace B). In the same Figure, it can also be observed that succinate was readily oxidized in the presence of 1 mM tellurite which completely blocks the oxidation of α-ketoglutarate.

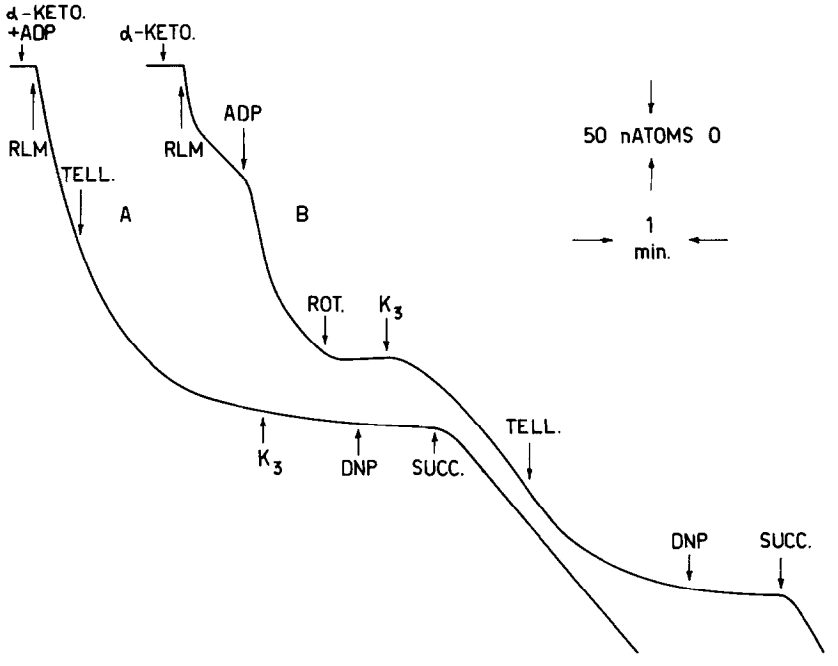


Figure 2: Oxidation of α-ketoglutarate by rat liver mitochondria (RLM).

Experimental conditions and incubation medium as in Table I.
Additions at the points indicated by arrows: liver mitochondria (7.5 mg protein) isolated in 0.25M sucrose according to Schneider and Hogeboom (5), 10 mM α-ketoglutarate, 1 mM ADP (trace A), 0.25 mM ADP (trace B), 1 mM $K_2TeO_3$ (tell.) (trace A), 2 mM $K_2TeO_3$ (trace B), 0.2 mM menadione ($K_3$), 0.1 mM 2,4-dinitrophenol (DNP), 10 mM succinate, 0.1 mM rotenone.

As Table II shows, the inhibitory effect of tellurite was completely reversed by addition of 5 mM dithioerythritol thus indicating that the action of tellurite is the consequence of its interaction with some mitochondrial SH groups.

It is very likely that through such an interaction tellurite modifies the conformation of NAD dehydrogenases preventing their association with NAD, or with the substrates, so that NAD reduction is consequently abolished. It is also probable that with the same mechanism, tellurite inhibits the "carriers" of the NAD dependent substrates preventing their accessibility to the respective dehydrogenases.

TABLE II

REVERSAL OF TELLURITE INHIBITION BY DITHIOERYTHRITOL

| Additions | $^{14}CO_2$ evolution CPM/mg prot. | |
|---|---|---|
| | - DTE | + DTE |
| (1-$^{14}$C) Pyruvate | 620 | 710 |
| (1-$^{14}$C) Pyruvate + tellurite 0.5 mM | 108 | 606 |

Rat liver mitochondria (5.3 mg protein) were suspended in a medium containing 10.4 mM $K_2HPO_4$, 2.4 mM $KH_2PO_4$, 9.6 mM NaF, 20.8 mM NaCl, 46.4 mM KCl, 4.8 mM $MgCl_2$, 3.5 mM ADP; final volume was 1 ml. Additions: 10 mM pyruvate 1-$C^{14}$ ($3.5 \times 10^5$ counts/min); 2.5 mM dithioerythritol (DTE).

Mitochondria were incubated for 10 minutes at 37°C and DTE was added after 150 seconds of incubation.

$^{14}CO_2$ was trapped by hyamine hydroxide and determined by liquid-scintillation counting.

Experiments with submitochondrial particles and isolated mitochondrial dehydrogenases are now in progress to clarify the mechanism of tellurite action.

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