COMPARISON OF THE EFFECTS OF ROTENONE AND AMYTAL ON MITOCHONDRIAL ELECTRON AND ENERGY TRANSFER AND TITRATION OF THE RESPIRATORY CHAIN WITH ROTENONE*

Lars Ernster, Gustav Dallner, and Giovanni Felice Azzone

Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden, and Institute of General Pathology, University of Padova, Padova, Italy.

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Fukami and Tomizawa (1956) and Lindahl and Öberg (1961) have reported that the fish poison, Rotenone, inhibits the mitochondrial oxidation of pyridine nucleotide-linked substrates but not that of succinate. Since this effect resembles the effect of Amytal (Ernster et al., 1955; Chance, 1956), a closer comparison of the action of two agents was considered to be of interest. It was found that Rotenone duplicates all the known features of the Amytal effect on electron transport in that a) it inhibits DPN-linked respiration of mitochondria and of submitochondrial preparations, but not soluble DPNH dehydrogenase or DPNH-cytochrome c reductase; b) it inhibits succinate-linked pyridine nucleotide reduction (Chance and Hollunger, 1957; Ernster, 196la,b, 1962); c) its effect on liver-mitochondrial DPN-linked respiration can be overcome by added vitamin K_3 (Conover and Ernster, 1962). However, Rotenone differs from Amytal in its action in two important respects:

1. Rotenone does not affect certain energy transfer reactions, which have been shown to be inhibited by relatively high concentrations (2-8 mM) of Amytal, namely: the mitochondrial P_i-ATP exchange (Löw et al., 1958) and ATPase reactions (Siekevitz et al., 1958; Löw, 1959); the phosphorylation coupled to the oxidation of succinate (Löw et al., 1955; Greengard et al., 1959; Azzone et al., 1961); and the relaxation of actomyosin fibers induced by sarcotubular ATPase (Azzone, 1961; Muscatello et al., 1962). A multiple mode of action of Amytal is also suggested by recent data of Chance et al. (1962).

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2. Whereas the effect of Amytal on DPN-linked electron transport is readily reversible and is only dependent on the concentration of the Amytal in the medium, Rotenone is firmly bound to mitochondria and submitochondrial particles, and the extent of the respiratory inhibition is dependent on the amount of Rotenone added per unit protein rather than its concentration. Data reported in Fig. 1 a illustrate this difference.

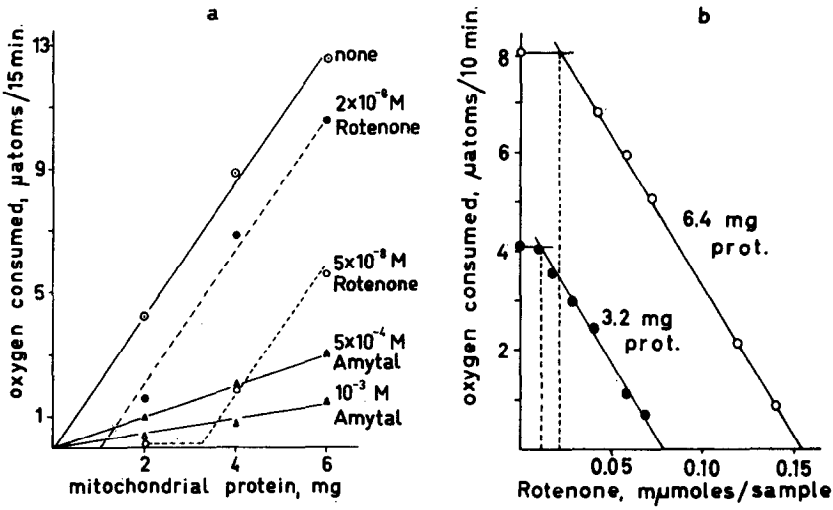

Fig. 1 a Effects of Rotenone and Amytal on Respiration of Rat Liver Mitochondria.

Each Warburg vessel contained 10 µmoles pyruvate, 10 µmoles L-malate, 50 µmoles P_i, pH 7.5, 16 µmoles $MgCl_2$, 2 µmoles ATP, 60 µmoles glucose, 150 KM units yeast hexokinase, 100 µmoles KCl, and 100 µmoles sucrose, in a final volume of 2 ml. Mitochondria, Rotenone (in the form of 0.02 ml ethanol solution), and Amytal were added in concentrations as indicated. Incubation at 30°C with air as the gas phase and 0.2 ml 2M KOH in the center well.

b Titration of "Rotenone-Sensitive Catalyst".

Conditions as in a. Rotenone and mitochondrial protein were added in amounts as indicated.

Owing to the latter property, Rotenone was suitable for titrating the mitochondrial respiratory chain (Fig. 1 b). In five experiments, with pyruvate + malate or glutamate as substrate, the amount of Rotenone required for complete inhibition of respiration in isolated rat liver mitochondria ranged between 24.7 - 28.0 mµmoles/g mitochondrial protein (Table 1). This value is about ten

times or more below that reported for any known electron transfer catalyst of the liver-mitochondrial respiratory chain, including pyridine nucleotides (Glock and McLean, 1956; Jacobson and Kaplan, 1957; Klingenberg et al., 1959), flavins (Chance and Williams, 1955), quinones (Green et al., 1956; Redfearn, 1961), and cytochromes (Chance and Williams, 1955; Schollmeyer and Klingenberg, 1962). It was also found to be about 2-3 times as low as the antimycin A titer (Estabrook, 1962) and more than 10 times lower than the oligomycin titer (Huijing and Slater, 1961) of the rat liver mitochondria.

Table I

Amount and Apparent Turnover Number of Rotenone-Sensitive Catalyst in Rat Liver Mitochondria.

The apparent turnover number has been calculated by dividing the oxygen consumption by the amount of Rotenone required between no and complete inhibition. The calculation rests on the assumption that each molecule of Rotenone blocks one molecule of "Rotenone-sensitive catalyst".

Expt. No.	Substrate	Oxygen consumption, μatoms/min./g protein	Amount of Rotenone required for complete inhibition, mμmoles/g protein	Apparent turnover number of "Rotenone-sensitive catalyst", $2e^-$ equiv./mole/min.
1	Pyruvate + L-malate	122	24.7	5400
2	" " "	124	27.3	6400
3	L-Glutamate	132	28.0	6700
4	"	140	25.4	5700
5	"	156	27.2	7500

From the titrations it was also possible to calculate the apparent turnover number of the "Rotenone-sensitive catalyst". This varied between 5400 and 7500 $2e^-$ equiv./mole/min. (Table I).

A striking difference between the action of antimycin A and oligomycin and that of Rotenone is apparent in the shape of the titration curves. Antimycin A and oligomycin described sigmoid types of titration curves, in accordance with previous data in the literature (Potter and Reif, 1952; Thorn, 1956; Estabrook,

1962; Huijing and Slater, 1961). In contrast, the extent of inhibition of respiration by any given amount of Rotenone was almost directly proportional to the total amount of Rotenone added. Hence it would appear that, whereas the antimycin A- and oligomycin-sensitive factors are present in the mitochondria at catalytic capacities which are in excess of the overall capacity of the respiratory chain, the Rotenone-sensitive factor constitutes the rate-limiting catalyst of the liver-mitochondrial DPNH oxidase system.

A detailed account of these results will appear elsewhere (Ernster et al., 1963).

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