THE INHIBITION OF NADH OXIDATION IN MAMMALIAN MITOCHONDRIA BY CHLORAMPHENICOL

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Chloramphenicol at concentrations of 6×10^{-6} to 3×10^{-5} M inhibits protein synthesis in bacteria (reviewed by Brock, 1961) and at similar concentrations inhibits protein synthesis by isolated mammalian mitochondria (Kroon, 1965). It has usually been reported that chloramphenicol inhibits protein synthesis in mammalian cells only at higher concentrations. Godchaux and Herbert (1966) found that chloramphenicol at concentrations above 10^{-3} M inhibited protein synthesis in erythroid cells but they suggested that this inhibition could be the result of an effect of the antibiotic on ATP formation. We have observed that chloramphenicol at concentrations above 3×10^{-4} M inhibits protein synthesis in Ehrlich Lettré ascites tumor cells and that this inhibition parallels the inhibition of respiration of the cells by the antibiotic (Haldar and Freeman, 1967). Both of these effects were reversed by glucose. Wenner (1965) observed that in ascites cells glucose reversed the inhibition of respiration caused by rotenone, an inhibitor of NADH dehydrogenase. Since glucose has a similar action both on chloramphenicol and rotenone inhibited respiration it seemed possible that chloramphenicol act at a site similar to rotenone. We report here that chloramphenicol at concentrations above 10^{-4} M inhibits NADH oxidation in mammalian mitochondria, perhaps as an inhibitor of NADH dehydrogenase.

METHODS

Rat liver mitochondria were isolated in the medium of Chance and Hagihara (1962) by the method of Hogeboom, Schneider and Pallade (1948). Beef heart mitochondria were isolated as described by Haas and Elliott (1963). Oxygen consumption was measured at 23° with a Clark type oxygen electrode from

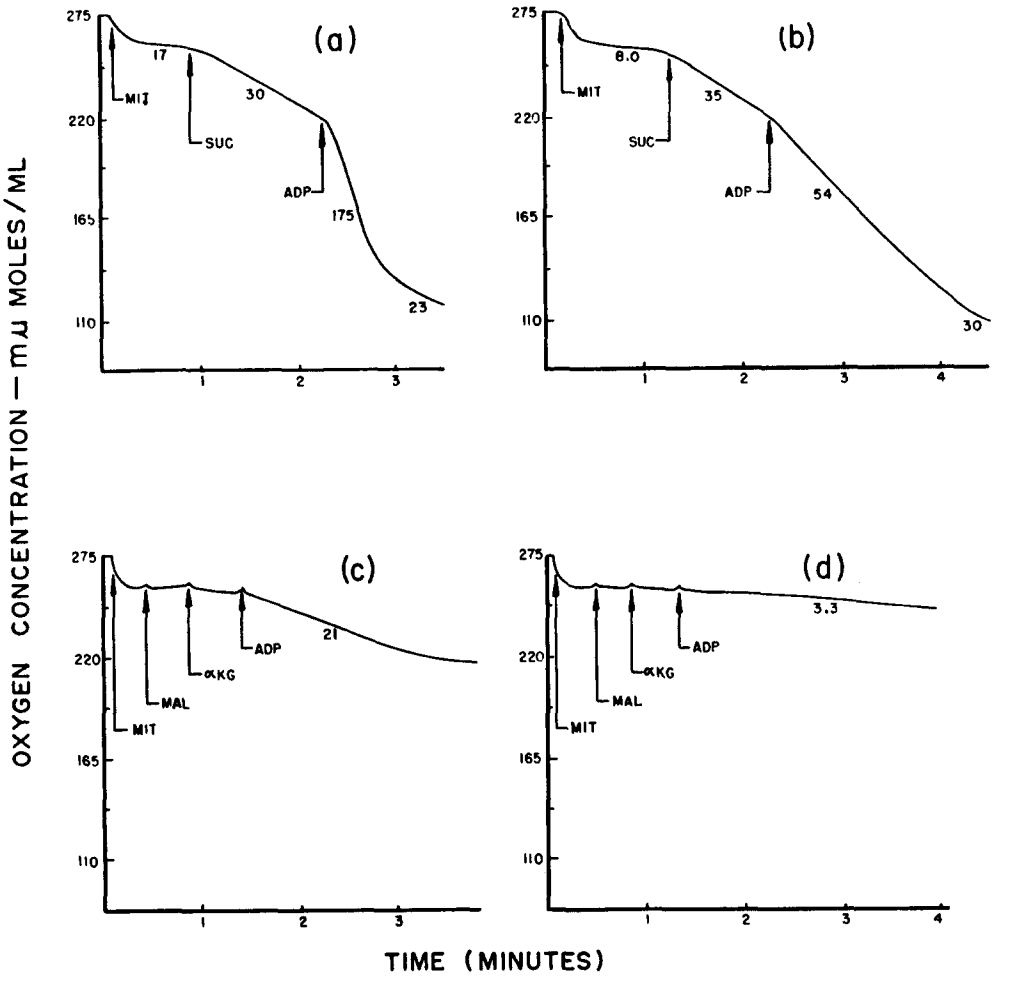

Fig. 1. The oxidation of succinate and α-ketoglutarate by rat liver mitochondria in the presence and absence of 6 x 10^{-3} M chloramphenicol (CAP): (a) succinate oxidation with no CAP, (b) succinate oxidation in the presence of CAP, (c) α-ketoglutarate oxidation with no CAP, (d) α-ketoglutarate oxidation in the presence of CAP. Incubations were in 3 ml and additions were as follows: mitochondria, 0.2 ml with 35 mg protein/ml, succinate 30 μmoles, α-ketoglutarate 30 μmoles, malonate 30 μmoles, ADP 750 mμmoles. The rates of respiration in units of mμmoles O_2/ml/min. are given below the electrode trace.

Yellow Springs Instrument Co. The incubation medium of Ziegler, et al. (1965) was used. Chloramphenicol (lots C237731 and H702103) was a gift from Parke, Davis and Co. Ltd.

RESULTS AND DISCUSSION

In Fig. 1a and 1b the oxidation of succinate by rat liver mitochondria in the presence and absence of 6×10^{-3} M chloramphenicol respectively is shown. It was observed that the antibiotic at this concentration did not inhibit the oxidation of succinate but caused a partial uncoupling. This effect was somewhat variable; the P:O ratio changed from an average of 1.63 to 1.14. The effect of chloramphenicol on the oxidation of α-ketoglutarate by rat liver mitochondria was strikingly different from the effect observed with succinate. A comparison of Fig. 1c and 1d shows that the oxidation of α-ketoglutarate was almost completely inhibited by 6×10^{-3} M chloramphenicol

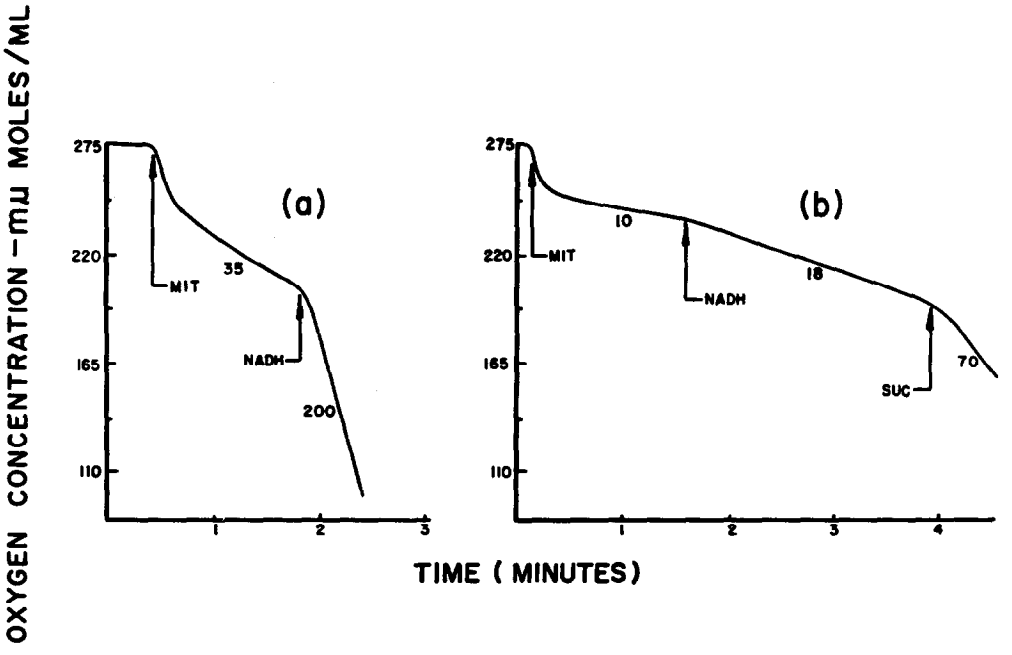

Fig. 2. The oxidation of NADH by beef heart mitochondria: (a) no chloramphenicol, (b) 6×10^{-3} M chloramphenicol. Additions were as follows: mitochondria, 0.2 ml with 18 mg protein/ml, NADH 6 μmoles, succinate 30 μmoles.

in tightly coupled mitochondria. The inhibition was not relieved by 10^{-4} M dinitrophenol. Chloramphenicol must therefore act between α-ketoglutarate and cytochrome b.

To determine if the antibiotic acted as an inhibitor of NADH oxidation, beef heart mitochondria were used since mitochondria from rat liver do not oxidize NADH (Lehninger, 1954). In Fig. 2a and 2b the results of tests on the oxidation of NADH by beef heart mitochondria are shown. It can be seen that 6×10^{-3} M chloramphenicol almost completely inhibited the oxidation of NADH but succinate was oxidized. At about 10^{-3} M chloramphenicol 50% inhibition of NADH oxidation was obtained.

We conclude that chloramphenicol at concentrations above 10^{-4} M inhibits the oxidation of NADH but not that of succinate. Chloramphenicol must therefore act at a site between NADH and cytochrome b, probably as an inhibitor of NADH dehydrogenase. This could not be confirmed by spectrophotometric examination of the oxidation state of the respiratory chain because of the absorption of ultraviolet light by chloramphenicol but experiments to further define the site of action of the antibiotic are in progress. Hodges and Hanson (1964) had previously reported that chloramphenicol acts as an uncoupling agent in maize mitochondria but they did not test for its effect on the oxidation of NADH.

There are many known inhibitors of NADH dehydrogenase (Chance and Hollunger, 1963). The present finding extends this list and indicates that many of the effects of the antibiotic on protein synthesis in mammalian cells (Godchaux and Herbert, 1966; Haldar and Freeman, 1967) are probably indirect. However, the concentrations that affect protein synthesis by isolated mitochondria (Kroon, 1965) are lower than those used here.

It would be of interest to determine which analogues of chloramphenicol are effective as inhibitors of protein synthesis in bacteria and as inhibitors of NADH oxidation in mammalian mitochondria. The L(+)threo isomer, which is inactive against bacteria, was reported by Godchaux and Herbert to inhibit protein synthesis in erythroid cells but we have not yet tried it in our

system. One further intriguing question is whether the anemia caused by chloramphenicol (Dameshek, 1960) could be accounted for by its action as a respiratory inhibitor.

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