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FUNCTIONAL AND STRUCTURAL CHANGES IN LIVER MITOCHONDRIA OF RATS DUE TO CCl_4 INTOXICATION—I

STUDIES ON STATE OF ELECTRON-TRANSPORT CHAIN

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Abstract—Functional changes in the electron-transport chain in the liver mitochondria of rats, induced by carbon tetrachloride treatment, have been studied in this work. Experimental evidence led us to the conclusion that along with uncoupling of oxidative phosphorylation, impairment of the respiratory chain is observed. The decrease in the rate of succinate oxidation is thought to be due to a deficit in cytochrome *c*. The marked respiratory inhibition observed during NAD-linked substrate oxidation is considered to be caused by impairment of either the first complex or the substrate dehydrogenases.

PREVIOUS investigations of alterations in the mitochondria of different organs caused by injection of CCl_4 into animals, have dealt, in the main, with their energy-synthesizing functions.[1,2] These observations have shown a decrease in production of ATP by the mitochondria, depending on the dose, intoxication duration, method of injection, etc. It should be pointed out that most of the data, obtained in the Warburg apparatus, were confirmed in Carafoli and Tiozzo's excellent studies conducted with the polarograph.[3] However, much less is known about electron-transport chain alteration produced by administration of CCl_4 to an intact animal. A greater lability of the respiratory chain during the oxidation of NAD-linked substrates than during succinate oxidation has been found.[4] The experiments described here present evidence of impairment of the electron-transport chain in rat liver mitochondria 24 hr after administration of CCl_4.

EXPERIMENTAL

Male albino rats of the Wistar strain were fasted overnight and then poisoned by CCl_4 (0·25 ml/100 g body wt.). A CCl_4 solution in mineral oil (1:1) was injected via gastric intubation. The animals were decapitated 24 hr later, and the removed liver was placed immediately in ice-cold 0·25 M sucrose solution with added 5 mM EDTA and 1 mM tris–HCl, pH 7·4. Mitochondria were isolated by the method described elsewhere.[5] The last elution and suspension procedures were carried out in sucrose with EDTA excluded. Mitochondrial suspensions containing 80–100 mg of mitochondrial protein per ml were kept at 0–4° and used in experiments not later than 2–3 hr after isolation. Respiration was determined polarographically with a stationary platinum electrode. Swelling was measured photometrically in the recording spectrophotometer CΦ-4A, at a wavelength of 520 nm. Base medium of incubation contained: 125 mM KCl + 20 mM tris–HCl, pH 7·4, respiratory substrates—5 mM, KH_2PO_4

— 5 mM. The details of the experiments are given in legends to the figures. Protein was determined by the Lowry method described elsewhere.[6]

RESULTS AND DISCUSSION

We conducted the initial procedures of mitochondria isolation in sucrose with 5 mM EDTA based on the data given in the work of Cohn *et al.*[5] concerning the accumulation of intracellular Ca^{2+} in mitochondria in the process of isolation. Mitochondrial elution and suspension were carried out in sucrose without EDTA.

Figure 1 shows a polarographic study of oxidation of NAD-linked substrates. The results indicate the manifestation of a marked respiratory rate decrease while using α-ketoglutarate, glutamate or pyruvate. In these experiments ADP addition did not accelerate the mitochondria respiratory rate and this led to the supposition that either uncoupling or the appearance of oligomycin-like affecting substances under CCl_4 influence was the reason. However, the addition of 2,4-dinitrophenol or Ca^{2+} (in any sequence) substances, which cancel the blocking action of oligomycin (and the agents, acting like oligomycin) had not increased O_2 consumption. Thus, in these experiments we have obtained completely uncoupled mitochondrial preparations. Nevertheless, the rate of oxidation of NAD-linked substrates was as little as 20–25 per cent of the respiratory rate of control mitochondria in state 3U according to Chance.[7] A search for the reasons for this oxidative capacity decrease was the purpose of further investigations.

In the system where cytochrome *c* did not induce respiratory acceleration, ascorbate + *N*,*N*-tetramethyl *p*-phenylendiamine (TMPD) addition produced an increase of

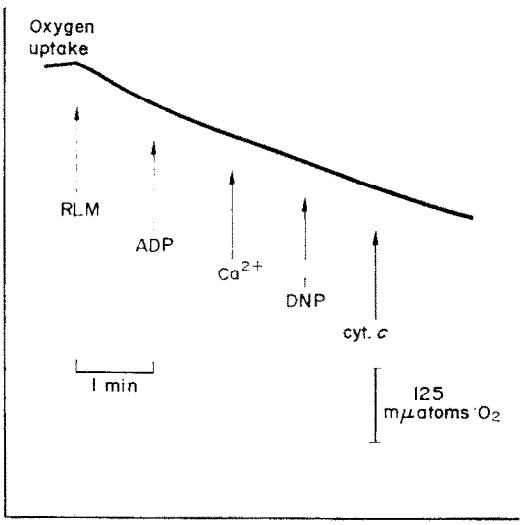

FIG. 1. Polarographic tracing showing NAD-linked substrates respiration in rat-liver mitochondria 24 hr after administration of CCl_4 (0·25 ml/100 g body wt.). The basic medium contained: 125 mM KCl, 20 mM tris buffer, (pH 7·4), 5 mM α-ketoglutarate, 1 mM NAD, 3 mM KH_2PO_4. When added, 200 μM ADP, 100 μM Ca^{2+}, 100 μM DNP, 10 μM cytochrome *c*. Final volume 1·3 ml. Amount of mitochondrial protein was 2 mg. Temperature, 22°.

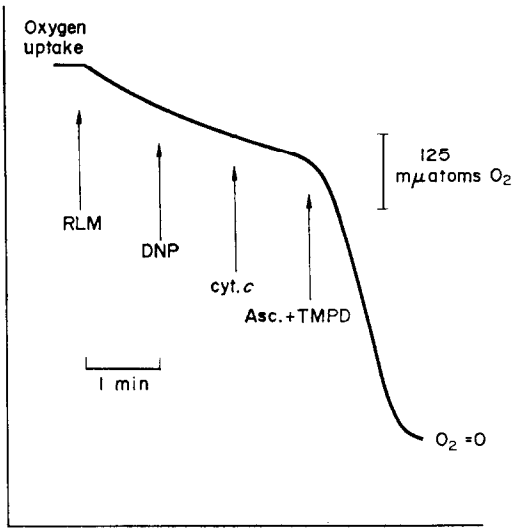

FIG. 2. Stimulation of respiration by ascorbate + TMPD in CCl_4-treated mitochondria. Basic medium and experimental conditions as in Fig. 1. When added, 100 μM DNP, 10 μM cytochrome *c*, ascorbate 10 mM, TMPD 50 μM.

O_2 consumption (Fig. 2). As cytochrome *c* is known to be the entering of electrons from TMPD,[8,9] this acceleration was indicative of normal functioning of respiratory chain carriers from cytochrome *c* to O_2.

If cytochrome *c* and succinate are added to liver mitochondria of poisoned rats, which oxidate NAD-linked substrates at a low rate, then the oxidation of succinate proceeds at a rate usual for the state 3U (Fig. 3). The succinate branch of the mitochondrial respiratory chain is probably more resistant to the injury induced by CCl_4;

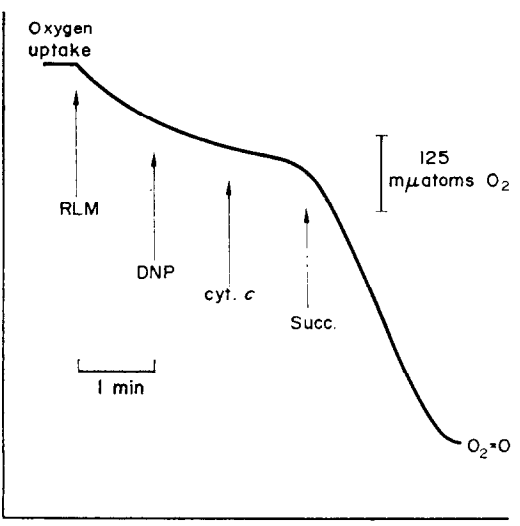

FIG. 3. Stimulation of respiration by succinate in CCl_4-treated mitochondria. Basic medium and experimental conditions as in Fig. 1. When added, 100 μM DNP, 10 μM cytochrome *c*, 3 mM succinate.

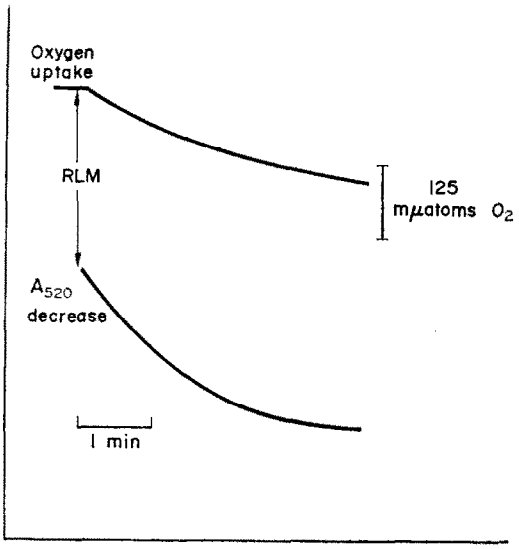

FIG. 4. Polarographic tracing and optical density decrease in rat-liver mitochondria following CCl^4 intoxication. Basic medium and experimental conditions as in Fig. 1.

cytochrome *c* leakage in our experiments is the only limiting factor of succinate oxidation. Taking into account the opinion of most authors about the presence of mutual respiratory carriers for the NAD-linked and succinate-linked chain, starting from coenzyme Q, the data illustrated in the figures lead us to conclude that localization of injury in the NAD-linked chain is higher than coenzyme Q (at the substrate chain end). As cytochrome *c* did not restore the oxidation rate of NAD-linked substrates, this can hardly be considered the main limiting factor of oxidation.

The results of these experiments have led to the assumption that the localization of the defect is either in the region of complex 1 or at the level of substrate dehydrogenases α-ketoglutarate, glutamate or pyruvate.

The investigation of the CCl_4-treated mitochondria in a cuvette with simultaneous recording of O_2 consumption and changes in optical density showed that together with disturbances revealed in substrate oxidation, alterations in mitochondrial volume could be observed, which are indicative of mitochondrial swelling (Fig. 4). As it is known that mitochondrial swelling may result in respiratory chain impairment,[10–12] it may be assumed that the manifested changes in the oxidative ability of the mitochondria are induced either by action of CCl_4 (or its metabolic product in the animal's organism) or by swelling, or by these factors combined. These studies are being carried out at present in our laboratory and will be reported in a separate communication.

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