The Effect of CoQ_{10} on Rat Liver Mitochondria Succinoxidase Activity in Carbon Tetrachloride Liver Injury

The distribution of various components of electron transport chains within the cell of fatty liver has been investigated by severals authors (BIAGGINI[1], WINTER[2,3]). The pyridine nucleotides (DPN-TPN) content of fatty liver due to CCl_4, white phosphorus and to a diet deficient in choline is strongly decreased and the distribution is much altered, because the pyridine nucleotides mitochondrial fraction decreases and supernatant fraction increases (DIANZANI[4]). A higher permeability of mitochondria from fatty liver exists also for cytochrome c. The cytochrome c content linked to mitochondria in fatty liver due to CCl_4 decreases, whereas that recovered in the supernatant fluid is increased (DIANZANI and VITI[5]); a similar decrease occurs for coenzyme A (SEVERI and FONNESU[6], HEIM et al.[7]). Recently CRANE et al.[8] isolated from beef heart mitochondria a water-insoluble quinone, now known as CoQ_{10}, that, at least in the oxidation of succinate, is on the main pathway in mitochondrial electron transport (LESTER and FLEISCHER[9]). In addition, AIYAR and SREENIVASAN[10] found that also the fraction of mitochondrial CoQ_{10} decreases while that in the supernatant correspondingly increases, in the case of CCl_4 poisoning. It therefore seems interesting to see whether the addition *in vitro* of CoQ_{10} to mitochondria from fatty liver influences respiratory activity.

Material and Methods. Albino rats (Wistar strain) fed on a mixed diet of bread and vegetables and weighing 150 to 200 g were used. Fatty infiltration of the liver was obtained either by subcutaneous injection of CCl_4 (0.2 ml of 20% olive oil solution) every day for five days, of by intraperitoneal injection of 0.2 ml of CCl_4, 24 h before death; in both methods the same type of behaviour was evident.

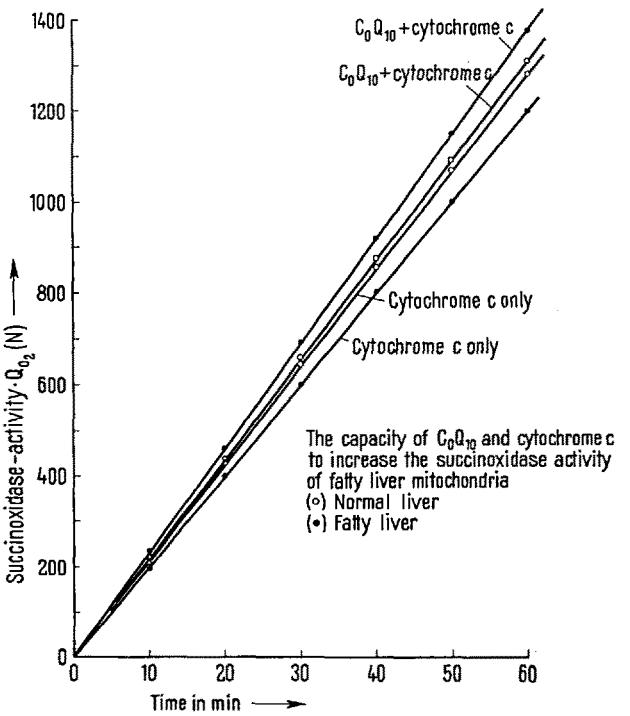
The liver was removed immediately after death, weighed and then kept at 2°C. 10% homogenates were prepared in a Potter-Elvehjem type homogenizer with 0.25 *M* sucrose. Mitochondria were obtained according to DIANZANI and SCURO[11]. Succinate oxidation was studied by measuring the oxygen uptake in a Warburg apparatus at 37°C. Oxygen consumption was followed for 60 min. The reaction system contained: 0.033 *M* Na_2HPO_4-KH_2PO_4 buffer, pH 7.4; $4 \times [10^{-4}]$ *M* $AlCl_3$, $6H_2O$; $4 \times [10^{-4}]$ *M* $CaCl_2$; when added, cytochrome c had a final concentration of 1.10^{-5} *M*; 0.02 *M* sodium succinate; 0.05 ml of an ethanolic solution of CoQ_{10} (final concentration 1×10^{-5} *M*); in the control flasks 0.05 ml of ethanol were added. The amount of mitochondria per flask corresponded to 20 mg wet liver. Final fluid volume was 3 ml. The central well of the Warburg flasks contained 0.2 ml of 30% NaOH. Gas phase was 100% oxygen. $QO_2(N)$ values (µl O_2/h/mg nitrogen) were calculated. Nitrogen determinations were made by a Microkjeldahl technique. The standard deviation and the 't' value of FISHER[12] were calculated. The differences between two averages considered significant corresponded to a probability $P < 0.01$. The crystalline CoQ_{10} used was partly kindly supplied by Dr. K. Folkers of Merck, Sharp and Dohme, and partly prepared by us by the method of CRANE, LESTER, WIDMER, and HATEFI[13]. In the experiments made with the two products no difference was noted. All other substances were Merck products.

Results and Discussion. The data reported in the Table and in the Figure show that, when CoQ_{10} is added to normal liver mitochondria, no considerable stimulation of oxygen uptake appears in the presence of cytochrome c;

Succinoxidase activity stimulation of fatty liver mitochondria by CoQ_{10} and cytochrome c

Additions	Normal liver mitochondria		Fatty liver mitochondria	
None	324.44 ± 126.31	(11)	282.58 ± 101.45	(10)
CoQ_{10}	308.40 ± 56.57	(6)	302.76 ± 57.52	(10)
Cytochtome c	1285.60 ± 300.00	(9)	1236.37 ± 82.66	(12)*
Cytochrome c + CoQ_{10}	1319.72 ± 289.25	(10)	1386.90 ± 70.99	(9)*

The data are expressed as µl of oxygen uptake/h/mg nitrogen ± σ standard deviation; the numbers of experiments are given in brackets. The two values marked with * differ significantly ('t' = 3,48 and $P < 0.01$).



[1] C. BIAGGINI, Arch. Sci. Biol. *35*, 418 (1951).
[2] I. C. WINTER, J. biol. Chem. *135*, ETA (1940).
[3] I. C. WINTER, J. biol. Chem. *142*, 17 (1942).
[4] M. U. DIANZANI, Biochim. biophys. Acta *17*, 391 (1955).
[5] M. U. DIANZANI and I. VITI, Biochem. J. *59*, 141 (1955).
[6] C. SEVERI and A. FONNESU, Proc. Soc. exp. Biol. Med. *91*, 368 (1956).
[7] F. HEIM, F. LEUSCHNER, and A. OTT, Arch. exp. Path. Pharmak. *229*, 360 (1956).
[8] F. L. CRANE, Y. HATEFI, R. L. LESTER, and C. WIDMER, Biochem. biophys. Acta *25*, 220 (1957).
[9] R. L. LESTER and S. FLEISCHER, Arch. Biochem. *80*, 470 (1959).
[10] A. S. AIYAR and A. SREENIVASAN, Biochem. J. *82*, 179 (1962).
[11] M. U. DIANZANI and S. SCURO, Biochem. J. *62*, 205 (1956).
[12] R. E. FISHER, *Metodi statistici ad uso dei ricercatori* (U.T.E.T., Torino 1948).
[13] F. L. CRANE, R. L. LESTER, C. WIDMER, and Y. HATEFI, Biochem. biophys. Acta *32*, 73 (1959).

an insignificant variation is observed when the substrate is incubated with CoQ_{10} without cytochrome c. In the case of mitochondria from fatty liver, on the other hand, significant increase in mitochondrial respiration is found when mitochondria are incubated with CoQ_{10} in presence of cytochrome c, while the increase is not statistically significant when cytochrome c is not added.

CRANE et al.[14] found that succinoxidase activity of electron transport particles prepared by isooctane and deoxycholate extraction was restored by addition of both CoQ_{10} and cytochrome c to the medium. The same behaviour is also observed in CCl_4 liver injury: while the addition of only CoQ_{10} to mitochondria of fatty liver does not stimulate respiration, the addition of CoQ_{10} in presence of cytochrome c stimulates significantly the O_2 uptake (the two values marked with * in the Table). The fact that those two values differ significantly might be interpreted in terms of an increased mitochondrial membrane permeability, as observed by several authors (DIANZANI[5,15]). This would bring about an easier access to the coenzymes, which are in excess in the reaction medium, to the sites suitable to their activity.

Riassunto. È stato studiato il comportamento dell'attività succinossidasica dei mitocondri di fegato di ratto normale e intossicato con CCl_4 in presenza di CoQ_{10}. Si è notato che l'aggiunta di CoQ_{10} in presenza di citocromo c ai mitocondri di fegato grasso stimula la respirazione, fatto che può essere interpretato come aumento di permeabilità della membrana mitocondriale.

ANNA CASU

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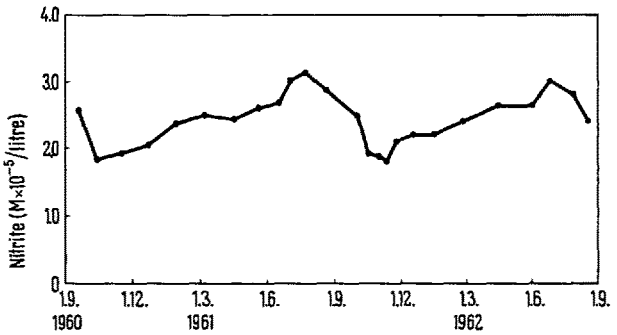
[14] F. L. CRANE, C. WIDMER, R. L. LESTER, and Y. HATEFI, Biochem. biophys. Acta *31*, 476 (1959).
[15] M. U. DIANZANI and U. MARINARI, Biochem. biophys. Acta *48*, 552 (1961).

Seasonal Changes in the Nitrate-Reducing Activity of a Green Alga[1]

Nitrate reduction *in vivo* by the green alga, *Ankistrodesmus braunii*, has been studied in some detail (for a summary, see [2]). In the course of this work we observed that, under constant experimental and growth conditions, the algae appeared to have a lower nitrate-reducing capacity in late autumn and early winter than in the rest of the year. A series of quantitative determinations of the activity *in vivo* of the systems reducing nitrate and nitrite, carried out during the past two years, has revealed the existence of pronounced seasonal changes in enzymatic activity. The results shown in the Figure for the reduction of nitrate have been obtained in the presence of $2 \times 10^{-3} M$ 2,4-dinitrophenol (DNP) at pH 6.5 in the dark. This concentration of DNP completely inhibits the further reduction of nitrite and has no influence on the reaction nitrate→nitrite[3]. Under constant conditions, a minimum of activity can be observed in October to November. In the course of winter and spring, the activity slowly rises to a maximum in June to July which, in turn, is followed by a rather sharp decline. During the summer maximum, the activity of the nitrate-reducing system is about 75% higher than in late autumn. The capacity of the system responsible for the reduction of nitrite was found to show changes quite closely resembling those of nitrate reduction. A similar behaviour with even greater seasonal changes has recently been found in the hydrogenase activity of intact cells of a strain of *Chlorella pyrenoidosa*[4]. Therefore it seems that periodic changes of enzymatic activities in the course of the year are quite a common phenomenon with green algae of those genera that are widely used for physiological and biochemical research.



Nitrate-reducing activity of intact cells of *Ankistrodesmus braunii* at different times of the year. Formation of nitrite in 2 h under standard conditions in the dark. Cell concentration, 0.8 mg dry weight/ml; phosphate buffer, pH 6.5; nitrate concentration, $1.7 \times 10^{-2} M$; DNP concentration, $2 \times 10^{-3} M$; temperature, 22°C.

Investigations on the reduction of nitrate and nitrite have also been carried out with cell-free extracts from *Ankistrodesmus*. For this work, *Ankistrodesmus braunii* (strain Marburg) was grown under continuous illumination (light intensity, 4000 lux) in a medium containing nitrate[5]. The cells were centrifuged, taken up in phosphate buffer of pH 6.5, and then broken by violent shaking with small glass beads in a Merkenschlager homogeniser (Type MSK; B. Braun, Melsungen) at 3–8°C. Cell walls and larger fragments were removed in a refrigerated centrifuge (Type Omikron; M. Christ, Osterode) at 2000 *g* and 4°C. The supernatant was found to contain both nitrate and nitrite-reducing enzymes. The nitrite reductase activity of this preparation could be inhibited by $2 \times 10^{-3} M$ DNP at pH 6.5. Thus the cell-free extracts were found to have the same sensitivity towards this inhibitor as was observed with intact cells. In addition, a separation of nitrate and nitrite reductases could be achieved by high-speed centrifugation at 4°C. After 4 h at 104000 *g*, the nitrite reductase activity was found in the sediment, whereas the nitrate reductase activity remained in the supernatant. Some other properties of the nitrate and nitrite reducing systems of *Ankistrodesmus* are summarized in the Table. They are in good agreement with those of the corresponding enzymes obtained from fungi, higher plants, and

[1] This work was supported by the Deutsche Forschungsgemeinschaft.
[2] E. KESSLER, Symp. Soc. exp. Biol. *13*, 87 (1959).
[3] E. KESSLER, Planta *45*, 94 (1955).
[4] E. KESSLER and W. LANGNER, Naturwiss. *49*, 331 (1962).
[5] E. KESSLER, W. LANGNER, I. LUDEWIG, and H. WIECHMANN, Plant and Cell Physiol., in press.