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SHORT COMMUNICATIONS

Functional and structural changes in liver mitochondria of rats due to CCl_4 intoxication–III. Studies of osmotic behaviour

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PHOSPHOLIPIDS in mitochondrial membranes has been shown to be important for a number of functional activities of mitochondria, such as in energy transformation,[1] function of succinate dehydrogenase,[2] cytochrome oxidase,[3] ion transfer[4] etc. Moreover, Scarpa *et al.*[4] and Rendi[5] showed that phospholipids were cultivated for maintaining the osmotic properties of mitochondria. The present paper shows some peculiarities in the osmotic behaviour of liver mitochondria of rats poisoned with CCl_4.

Rats of the Wistar strain were poisoned and liver mitochondria prepared as described previously.[6] In some experiments liver mitochondria of control rats were exposed to treatment with acetone + H_2O (90/10, v/v) and ammonium for phospholipid extraction according to the method Fleischer *et al.*[7] Mitochondrial osmotic behaviour was followed by the photometric method described by Tedeschi and Harris.[8] The mitochondrial protein was determined by the method of Lowry *et al.*[9]

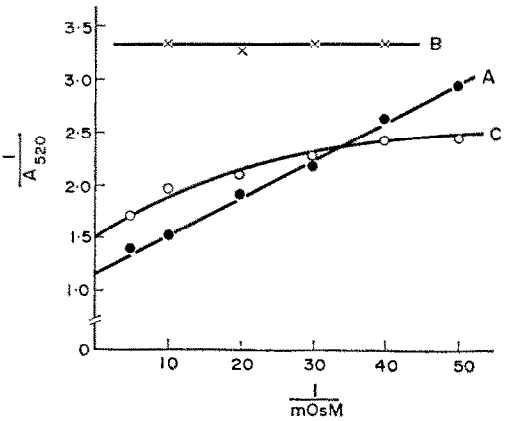

FIG. 1. Osmotic behaviour of liver mitochondria in KCl solutions of different tonicity. ●——● = control mitochondria (1 mg protein/ml). ×——× = phospholipid-depleted mitochondria (0·4 mg protein/ml). ○——○ = CCl_4-mitochondria (1·2 mg protein/ml). The abscissa is the reciprocal of KCl solutes osmolarity, the ordinate is the reciprocal of the mitochondrial suspension absorbance. Medium: KCl in indicated concentrations, 5 mM Tris–HCl, pH 7·4, rotenone, 6×10^{-6} M.

Liver mitochondria of control rats behave as perfect osmometers when suspended in KCl solutions with rising osmolarity (Fig. 1, line A). The depletion of phospholipids leads to a loss of osmotic properties of mitochondria (Fig. 1, line B). Figure 1, line C shows that CCl_4 leads to a marked change in the osmotic behaviour of mitochondria of poisoned rats.

Tedeschi and Harris[8] described the phenomenon of osmotic reversal of swelling by the addition of nonpenetrating ions solutions to the mitochondria. Analogous results were obtained by Bangham *et al.*[10] in experiments with artificial phospholipid vesicles. Figure 2 shows that control rat liver mitochondria possess the ability to change their osmotic-dependent space, responding on the increase of KCl concentrations in the medium, in accordance with Boyle–Van Hoff's law. In contrast to this, liver mitochondria from poisoned rats do not change their volume under the same conditions. The inability of liver mitochondria of rats poisoned with CCl_4 to obey Boyle–Van Hoff's law is probably

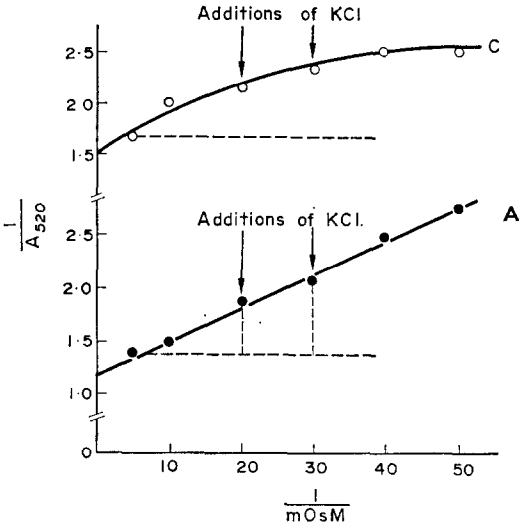

FIG. 2. Osmotic reversal of mitochondrial hypotonic swelling. Designations and conditions as in Fig. 1. The arrows show the additions of concentrated KCl solution up to 200 mOsmoles tonicity. Concentrated KCl solution was added in volume 0·05–0·1 ml for lessening the effect of dilution. The horizontal dashed lines show the predicted reversal, the vertical dashed lines show the experimental reversal. The experimental reversal of CCl_4-mitochondria was absent.

caused by the increase in the passive ion movement through the damaged membrane which becomes comparable with the rate of water movement.

Experiments of Scarpa and Azzone[4] on the restoration of the osmotic characteristic of liver mitochondria by the addition of single phospholipids, also Rendi[5] and Bangham *et al.*[10] on the osmotic behaviour of artificial phospholipid vesicles have shown that the semi-permeable properties of the membranes to a large degree are dependent on their phospholipid component. From this point of view one can conclude that the described changes in the osmotic behaviour of liver mitochondria are due to the interactions of toxic metabolites caused by CCl_4 with mitochondrial phospholipids, probably with phosphatidyl, ethanolamine and phosphatidyl inositole in particular.

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REFERENCES

1. C. R. ROSSI, C. S. ROSSI, L. SARTORELLI, D. SILIPRANDI and N. SILIPRANDI, *Archs Biochem. Biophys.* **99**, 214 (1962).
2. R. L. LESTER and S. FLEISCHER, *Biochim. biophys. Acta* **47**, 358 (1961).
3. G. P. BRIERLEY and A. J. MEROLA, *Biochim. biophys. Acta* **64**, 205 (1962).
4. A. SCARPA and G. F. AZZONE, *Biochim. biophys. Acta* **173**, 78 (1969).
5. R. RENDI, *Biochim. biophys. Acta* **135**, 333 (1967).
6. V. V. LYACHOVICH, V. M. MISHIN, A. V. DOLGOV and I. B. TSYRLOV, *Biochem. Pharmac.* **20**, 1437 (1971).
7. S. FLEISCHER, B. FLEISCHER and W. STOECKENIUS, *J. Cell Biol.* **32**, 193 (1967).
8. H. TEDESCHI and D. L. HARRIS, *Biochim. biophys. Acta* **28**, 392 (1958).
9. O. H. LOWRY and J. A. LOPEZ, *J. biol. Chem.* **162**, 426 (1946).
10. A. D. BANGHAM, J. DE GIER and G. D. GREVILLE, *Chem. Phys. Lipids* **1**, 225 (1967).