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A RAPID PROCEDURE FOR THE ISOLATION OF RAT LIVER MITOCHONDRIA

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SUMMARY

A simplified procedure for the isolation of rat liver mitochondria is described. The procedure yields well preserved and functionally active mitochondria, as assessed by electron microscopy and the phospholipid–protein ratio, oxidative phosphorylation, and swelling.

INTRODUCTION

A method has been developed for the isolation of mitochondria from rat liver involving homogenization and filtration with sand and having the advantage of shortening the preparation time.

MATERIALS AND METHODS

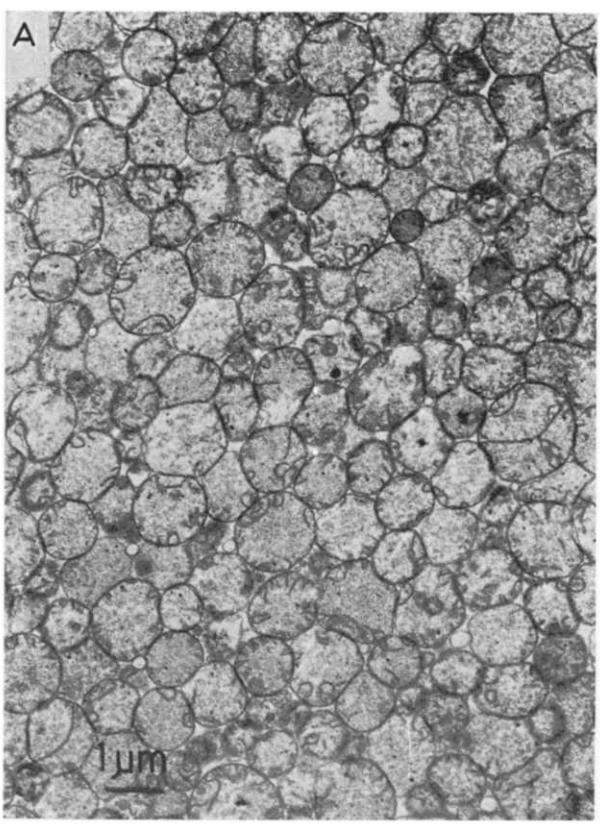
The livers of adult male rats (Wistar strain, obtained from the Instituto Gulbenkian de Ciência, Oeiras, Portugal) killed by decapitation were rapidly excised, weighed, minced with scissors, and immersed in the isolation medium (0.33 M sucrose, 0.025 mM EDTA (sodium salt), and 0.015 M Tris, pH 7.4) at 0°. Subsequent operations were performed at 0–4°. The minced tissue was homogenized (about 5 min) in a mortar with 12 times its weight of sand, which had been pre-cooled to −20°. The mixture was immediately filtered through glass wool with 10 vol. of the isolation medium and centrifuged at 800 × *g* for 10 min (Lourdes 9RA rotor). The supernatant was decanted, centrifuged at 8200 × *g* for 10 min, and the resulting pellet resuspended in 0.33 M sucrose. The mitochondria were collected from this suspension by recentrifugation at 8220 × *g* for 10 min. It appears that the filtration step "clears" the homogenate, making multiple centrifugation steps unnecessary, therefore reducing the processing time and allowing experiments to be carried out with "fresher" mitochondria. The procedure generally yields about 15 mg protein per g liver (wet weight).

Preparation of the sand

Quartzitic sand, which had been sifted through a 500-mesh sieve, was treated with conc. HCl for 2 h at 60°. Excess acid was subsequently removed by washing with tap water, with shaking. The sand was then mixed with 3 parts of 0.02 M EDTA (sodium salt), and heated for 30 min at 80°; this step was repeated 2 more times. Finally the sand was washed 6 times with deionized and redistilled water for 30 min each time. A large quantity of prepared sand was stored at —20°. Immediately prior to commencing the homogenization procedure, an aliquot was transferred to a chilled mortar.

Analytical procedures

Phospholipids were extracted[1], assayed for phosphorus, and chromatographed together with reference compounds on activated (1 h at 110°) Silica gel G (E. Merck) plates. Chroroform–methanol–acetic acid–water (125:37:10:2, by vol.) was used as solvent system. Lipid material was detected with iodine vapours[2], phosphate groups with the reagent according to DITTMER AND LESTER[3], and amino groups with a 0.5 % ninhydrin solution in 85 % butanol. The relative proportions of the phospholipids were estimated by phosphorus assay of the eluates from the spots which had been detected with iodine. Oxidative phosphorylation was estimated as P/O ratio by the conventional manometric technique[4]. Phosphate-induced swelling was



followed by measurement of the absorbance at 520 nm[5]. Glucose-6-phosphatase (EC 3.1.3.9) was assayed as described by DE DUVE *et al.*[6]. Cholinephosphotransferase (EC 2.7.8.2) activity was determined as described by SARZALA *et al.*[7]. Protein was determined using the procedure of LOWRY *et al.*[8], and phosphorus as described by FISKE AND SUBBAROW[9].

RESULTS AND DISCUSSION

The phospholipid content of the rat liver mitochondria prepared by the above technique was 0.182 μg atom of phospholipid phosphorus per mg of protein, which is within the range of values found by others[10–12]. The relative amounts of the various types of phospholipids were also in agreement with values found in the literature for mitochondria prepared by other techniques[13–14]. Cardiolipin P–phosphatidylcholine P–phosphatidylethanolamine P were found to be in the molar proportions of 1:3.8:3.6. The P/O ratios obtained with glutamate, α-ketoglutarate, β-hydroxybutyrate, and succinate were 2.96 $\pm$ 0.14, 3.39 $\pm$ 0.20, 2.67 $\pm$ 0.16, and 1.71 $\pm$ 0.18, respectively. Swelling of the mitochondria was readily demonstrated following incuba-

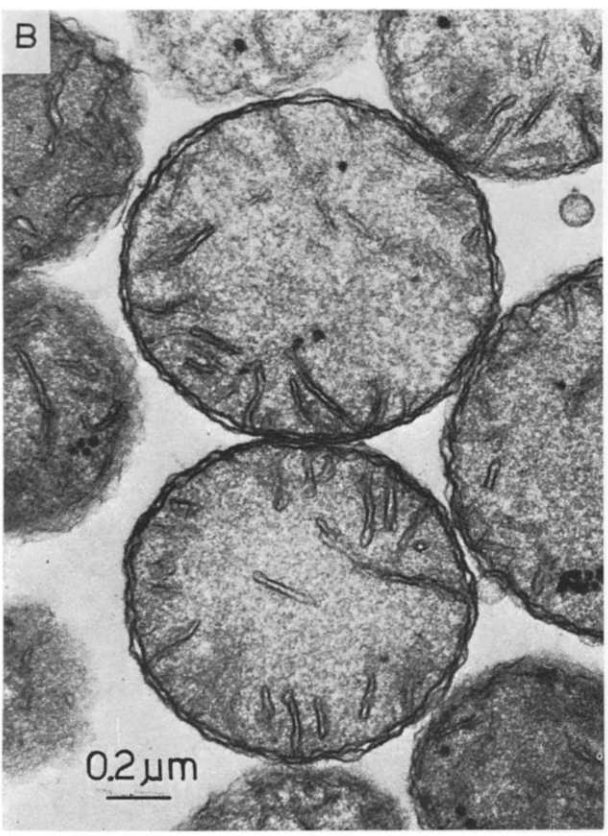


Fig. 1. Electron micrographs of rat liver mitochondria. Fixation with osmium tetroxide[16], embedding in Epon[17]. Sections were stained with uranyl acetate and lead citrate[18]. A. Low magnification. B. High magnification.

tion with phosphate, as reported for other rat liver mitochondrial preparations[15]. Ultrastructural examination showed well preserved mitochondria, with intact membranes and a dense matrix (Figs. 1A and 1B). Low contamination with microsomes, glycogen and lysosomes was suggested by study of the electron micrographs. Glucose-6-phosphatase determinations indicated a 5 % contamination with microsomes, and cholinephosphotransferase measurements indicated a microsomal contamination of 2.3 %; this compares favourably with values obtained for mitochondria isolated by other techniques[7,19].

The morphological, chemical, biochemical and physiological parameters described above show that the procedure reported here affords a rapid means for obtaining structurally and functionally intact mitochondria.

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