THE EFFECT OF AGE ON MITOCHONDRIAL ULTRASTRUCTURE AND ENZYMES

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SUMMARY

The ultrastructure of perfused livers and of mitochondrial fractions from 6 months and 30 month-old C57/BL mice were studied. In old mice the liver cell mitochondria were enlarged and rounded with a light "foamy", vacuolated matrix, short cristae and a loss of dense granules. Quantitative studies showed a 60% increase in the mean size and an increased proportion of larger mitochondria in intact 30 month-old perfused livers. Endothelial and Kupffer cell mitochondria were smaller than those of the parenchymal cells.

Mitochondria in pellets prepared from 6 and 30 month-old livers were rounded and condensed although there were a few larger and "foamy" mitochondria in the preparations from old mice. Up to 47% of large mitochondria in the old livers were lost during cell fractionation. The levels of cytochrome oxidase and malate dehydrogenase were slightly decreased with age but their cytochemical localization was unchanged.

INTRODUCTION

There is considerable evidence to suggest that there are mitochondrial changes in ageing and in tumours (1-9) but there are few detailed studies. In this preliminary paper we review the problem and describe age-associated changes in mitochondria in the mouse liver in our colony of ageing mice. We also attempt to make a correlation between structural and functional changes in mitochondria.
MATERIALS AND METHODS

The livers from 6 month-old and 30 month-old male C57BL/ICRF/a<sup>t</sup> mice were compared. For morphology the livers were perfused with Waymouth's medium and 2.5% glutaraldehyde in 0.2 M cacodylate buffer. Mitochondrial fractions were prepared from liver homogenates by a modification of Schneider's method (10). The pellets were then fixed in 2.5% glutaraldehyde or used for biochemistry. All specimens for morphology were post-fixed in Palade's fluid, dehydrated in graded alcohols and embedded in Araldite using epxoy propane as transitional solvent. Uranyl acetate and lead citrate stained sections were viewed under an Hitachi HS7S or Siemens 1 electron microscope. For cytochemistry, the livers were fixed for 1 hr in 0.1 M phosphate buffered 4% formaldehyde, or for 30 min in 1.5% glutaraldehyde in 0.2 M cacodylate buffer. Tissues were then rinsed in the appropriate buffer and chopped into 50 to 100 slices, and incubated in media for the demonstration of cytochrome oxidase (11) or malate dehydrogenase (12) for 45 min at 37°C. After rinsing OSO<sub>4</sub> post-fixation and rapid alcohol dehydration, the tissues were embedded in Spurr low viscosity embedding medium (13). Cytochrome oxidase and malate dehydrogenase activities were assayed spectrophotometrically in total liver homogenates and in mitochondrial preparations (14,15). The number and size of mitochondria were measured using a modification of the techniques described by Weibel (16) and Berger (17).

RESULTS

Morphology of Perfused Young and Old Liver Mitochondria

There were obvious morphological differences between the mitochondria of young (6 month) and old (30 month) mouse livers which had been similarly "well" fixed by perfusion with glutaraldehyde and postfixing with OSO<sub>4</sub>.

In the parenchymal cells the mitochondria of young mice appeared to be smaller and perhaps more plentiful in number. Although there was some variation in shape, the majority of mitochondria in young liver were elongated and rod-like (the "classical" mitochondrial structure) while old liver mitochondria were mostly rounded and enlarged. The degree of enlargement was not uniform but depended on the region of the lobule. The largest mitochondria were found in the central region of the lobules. The arrangement and number of cristae varied but the cristae in young liver mitochondria tended to extend further into the matrix and showed a more regular arrangement than those in the old mitochondria. In the old mitochondria cristae tended to be short and irregularly