Mitochondrial enzyme activity during in vitro ageing of human diploid fibroblasts

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At early CPD levels, the energy requirements of cultured human diploid fibroblasts (HDF) are met almost exclusively by glutamine oxidation and anaerobic glycolysis, the relative rates of glutamine and glucose utilization being subject to reciprocal regulation (Zielke et al., 1978; Sumbilla et al., 1981). With ageing in vitro, there is a highly significant shift to glycolysis (Bittles & Harper, 1984) and it has been suggested that this may indicate a decrease in oxidative phosphorylation caused by reduced structural and/or functional integrity of the mitochondria (Bittles & Samby, 1986). To assess the possible effects of ageing in vitro on HDF mitochondrial enzyme activity, the specific activities of three representative enzymes were determined at four stages during the cellular life-span in culture.

Human diploid embryonic lung fibroblasts, strain 2002 (Flow Labs), were roller cultured as previously described (Bittles & Harper, 1984). The life-span in vitro of this cell strain has been defined as 60 ± 3 CPD. Cells were harvested at CPD 22, 33, 43 and 55 using trypsin/EDTA, washed three times with PBS ‘A + B + C’, resuspended in 0.03 m-phosphate buffer pH 6.8, and disrupted by maximum amplitude sonication at 0°C for 3 × 10 s. Particulate material was removed by centrifugation, 12000g for 15 min at 4°C. O-S ATPase activity was measured by the method of Pullman & Penefsky (1963); GDH by that recommended by the D.G.K.C. (1974) and MDH activity according to Bergmeyer, U. (1974) Methods of Enzymatic Analysis, Academic Press, London and New York.

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Abbreviations used: HDF, human diploid fibroblasts(s); CPD, cell population doubling(s); PBS, phosphate-buffered saline; O-S ATPase, oligomycin-sensitive ATPase; GDH, glutamate dehydrogenase; MDH, malate dehydrogenase.


Between CPD 22 and 55 no significant changes were observed in the specific activities of either O-S ATPase, a mitochondrial inner membrane enzyme, or MDH, located in the matrix (Table 1). However, there was a highly significant, age-related increase in the activity of the matrix enzyme GDH ($P < 0.0001$).

In an earlier study, HDF intracellular glutamine concentration was shown to increase concomitantly with advancing CPD in culture, ascribed to the general reduction in metabolic activity characteristic of ‘older cells’ (Sambuy & Bittles, 1982). The GDH results obtained in the present investigation suggest that this interpretation requires revision: the increased intracellular glutamine concentration more probably reflected a primary, age-related decline in glutamine oxidation. Although the precise nature of the putative defect(s) associated with this change remains to be elucidated, structural and compositional changes in HDF mitochondrial membranes have been demonstrated by electron microscopy (Johnson, 1984) and immunoblotting (Ghadiminejad et al., 1987). As glutamine provides 30–50% of the energy requirements of HDF at low CPD (Zielke et al., 1984), any perturbation in the availability of the amino acid for energy provision must have profound effects at the cellular level. In particular, the switch to glycolysis previously observed may result in reduced availability of glucose as a source of ribose moieties for nucleic acid biosynthesis.

### Table 1. Mean specific activities of mitochondrial enzymes during in vitro ageing

<table>
<thead>
<tr>
<th>CPD</th>
<th>O-S ATPase (unit/mg protein)</th>
<th>Malate dehydrogenase (unit/mg protein)</th>
<th>Glutamate dehydrogenase (unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>63.1</td>
<td>291</td>
<td>3.8</td>
</tr>
<tr>
<td>33</td>
<td>61.6</td>
<td>270</td>
<td>3.7</td>
</tr>
<tr>
<td>43</td>
<td>57.9</td>
<td>240</td>
<td>6.6</td>
</tr>
<tr>
<td>55</td>
<td>64.7</td>
<td>298</td>
<td>10.6</td>
</tr>
</tbody>
</table>

Age-dependent loss of a mitochondrial antigen in cultured human diploid fibroblasts

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Introduction

The presence of antimitochondrial antibodies (AMA) in the sera of patients with primary biliary cirrhosis (PBC) is characteristic of this disease (Munoz et al., 1981). However, there is some diversity between patients as to the mitochondrial antigens against which these AMA react (Baum & Palmer, 1985). The majority of patients show reactivity on immunoblots against a major antigenic peptide (‘M2’) of Mr 74 kDa (for bovine heart mitochondria) together with a number of less prominent bands, frequently including relatively strong ones of Mr 54 and 43 kDa (Frazer et al., 1985; Lindenborn-Fotinos et al., 1985). The molecular mass of the major band is species-dependent but, within a given species, organ-independent (Ghadiminejad & Baum, 1987a). However, a minority of patients, although clinically indistinguishable from the others, show reactivity predominantly against an antigen (‘M4’) of Mr 52 kDa. (Lindenborn-Fotinos et al., 1985; Ghadiminejad & Baum, 1986). In this case the size of the antigen is apparently species-independent (Ghadiminejad & Baum, 1986).

Abbreviations used: AMA, antimitochondrial antibodies; PBC, primary biliary cirrhosis; ELISA, enzyme-linked immunosorbent assay.

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