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

CPD	O-S ATPase (unit/mg protein)	Malate dehydrogenase (unit/mg protein)	Glutamate dehydrogenase (m unit/mg protein)
22	63.1	291	3.8
33	61.6	270	3.7
43	57.9	240	6.6
55	64.7	298	10.6

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.

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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 M_r 74 kDa (for bovine heart mitochondria) together with a number of less prominent bands, frequently including relatively strong ones of M_r 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, 1987*a*). However, a minority of patients, although clinically indistinguishable from the others, show reactivity predominantly against an antigen ('M4') of M_r 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.

The precise identity of these various antigens is still a mystery. The major ('M2') antigens are normally, but not exclusively, associated with the inner mitochondrial membrane (Ghadiminejad & Baum, 1987*b*). The 52 kDa peptide ('M4') has been less extensively studied, but may be associated with the outer mitochondrial membrane (Ghadiminejad & Baum, 1986). Whatever their identity, the cross-reactivity of these antigens from species as diverse as yeasts, insects and man (Baum & Palmer, 1985) points to a degree of conservation compatible with some key cellular or developmental role(s). Because of this, and since a change in mitochondrial activity has been implicated in the process of ageing (Harper *et al.*, 1987), we have examined the reactivity of homogenates of cultured human diploid fibroblasts of different cell population doubling levels (CPD) against the AMA of marker PBC sera, of the common (74 kDa reactive-'M2') and less common (52 kDa reactive-'M4') type, to determine if any age-related difference in reactivity could be detected.

Materials and methods

The 'M2' and 'M4' sera used were from patients with clinically defined, stage-three PBC, and exhibited reactivities, in all immunological tests, fully characteristic of the 'M2' and 'M4' classifications respectively.

Human diploid fibroblasts were roller cultured, harvested and sonicated at CPD 22, 33, 43 and 55 (Harper *et al.*, 1987). Quantitative ELISA, cellular immunofluorescence

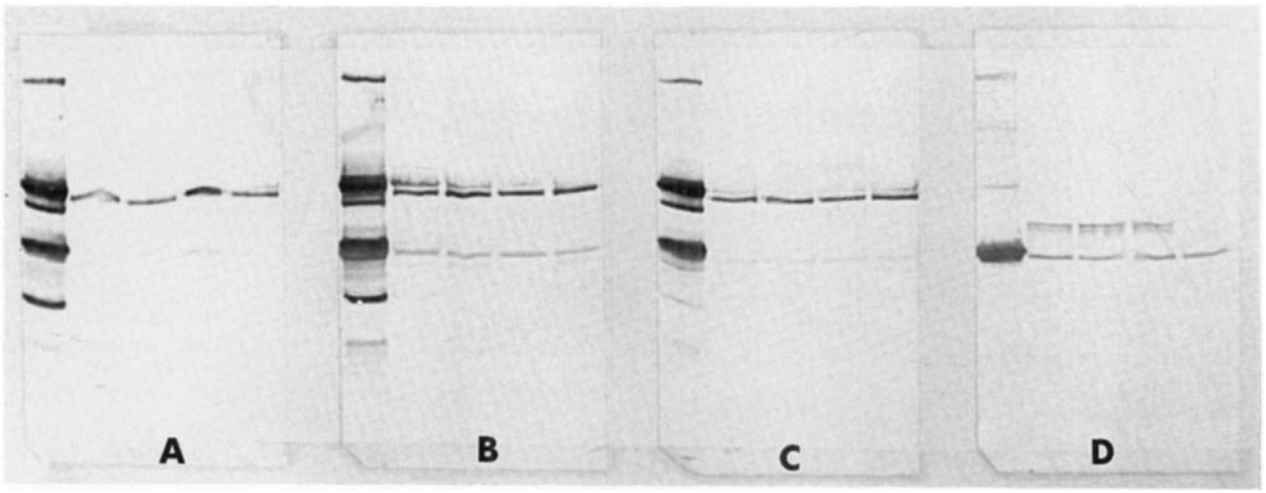

Fig. 1. *Immunoblots of 'mitochondrial' antigens in fibroblasts of different ages, detected by different PBC sera*

Each slab of five tracks represents (from left to right) antigens of control beef heart mitochondria, and sonicates of cultured human diploid fibroblasts of CPD 22, 33, 43 and 55, respectively. Slabs A, B and C were developed with marker PBC sera for the common, 'M2' pattern of antigens. The PBC serum used on slab D was that reacting with the rarer 'M4' antigen.

and immunoblotting were carried out as previously described (Ghadiminejad & Baum, 1987*b*).

Results and discussion

Quantitative ELISA. No significant differences were detected between the reactivities of sonicates from the four generations of cells against either of the PBC sera.

Immunofluorescence. Coded samples could, subjectively, be sorted into groups corresponding to differing CPD on the basis of the pattern of faint cellular immunofluorescence with the 'M2' serum. However, there was no single characteristic that clearly varied with ageing, and these observations may therefore not be significant.

Immunoblotting (Fig. 1). Immunoblots of three marker sera of the 'M2' classification revealed, for all four sonicates, a major band at a slightly lower molecular mass than that of the 74 kDa band for the control beef heart mitochondria. Fewer antigens were identified in the sonicates than in the control mitochondria, but no major difference was revealed by the three sera between sonicates of cells at different CPD. The 'M4' serum detected an antigen of 52 kDa in all five samples, and also (and quite reproducibly) a hitherto unrecognized doublet of extra bands in three of the sonicates, but not in that of the cells at CPD 55. When sera were absorbed with beef heart mitochondria, all bands disappeared (results not shown). Whatever their subcellular origin, therefore, these new 'M4' reactive bands seem to belong to the family of cross-reactive mitochondrial antigens. It remains to be seen whether their disappearance in the aged cells is a genuine, reproducible marker for cellular ageing and, if so, what might be the molecular significance of their loss in the ageing process.

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Changes in the relative proportions of creatine kinase–MM isoforms following eccentric exercise

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A large increase in serum creatine kinase (CK) activity usually indicates myocardial or skeletal muscle damage.

Abbreviations used: CK, creatinine kinase; MM, CK–MM isoform; ANOVA, analysis of variance.

Recently, isoelectric focusing has been used to observe the creatine kinase–MM isoform pattern following myocardial infarction (Morelli *et al.*, 1983) and in exercise-induced muscle damage (Clarkson *et al.*, 1987). In both cases, the three MM isoforms detected increased and then decreased in a sequence from MM1 through to MM3. It has been suggested that the MM1 : MM3 ratio is a more sensitive indicator of myocardial infarction than total CK, since it is elevated within a few hours of infarct, when total CK has not substantially increased (Morelli *et al.*, 1983).

The object of this study was to follow the course of CK–MM isoform release in exercise-induced muscle damage