REVERSAL OF AGE-DEPENDENT DECLINE IN RESPIRATORY CONTROL RATIO BY HEPATIC REGENERATION

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1. Introduction

Mature rats (3–12 months) survive the surgical removal of 2/3rds of the liver and that the remaining tissue regenerates to approximately the original size in 7 days [1]. Senescent rats (27–33 months) respond to partial hepatectomy in a similar manner although regeneration takes 3 times as long. This facility to stimulate rapid cell division in a tissue in which cells normally divide only rarely provides an opportunity to gain an insight into the mechanisms of age-related changes. Ultrastructural analysis of rat liver indicated that age-related phenomena may be affected by regeneration [2]. We have shown an age-dependent decline in respiratory control ratio [3] and an increase in permeability of the inner mitochondrial membrane [4]. This paper reports that hepatic regeneration resulted in the reversal of these age-dependent phenomena.

2. Experimental

Mature (3–12 months) and old (27–33 months) Wistar rats were bred in this department. Maintenance of the ageing colony was as in [5]. Partial hepatectomy was performed according to [1]. Rats were killed by cervical fracture and liver mitochondria isolated rapidly by a standard procedure [6] in a medium containing 250 mM sucrose, 5 mM Hepes (2-[*N*-2-hydroxyethyl piperazine-*N*-yl]-ethane sulphonic acid) and 0.5 mM EGTA adjusted to pH 7.5 with NaOH. Oxygen uptake was measured by use of a Clark-type electrode in a 5 ml glass reaction chamber fitted with a water jacket and magnetic stirrer. The medium for the assay of respiratory control ratios (RCR) and uncoupled rates of respiration contained 4–6 mg mitochondrial protein, 250 mM sucrose, 2.5 mM KH_2PO_4/NaOH (pH 7.5), 1.0 mM $MgCl_2$ and 5 μg rotenone in a final volume of 4.2 ml and was maintained at 25°C (pH 7.5) with rapid stirring. The reaction was started by the addition of 2.5 mM sodium succinate and followed by the addition of 0.75 μmol ADP/NaOH (pH 7.5) (state 3 rate). RCR was calculated from the linear state 3 rate of respiration divided by the linear rate of respiration following the exhaustion of ADP. Uncoupled rates of respiration were measured following the addition of 0.3 μM FCCP (carbonyl cyanide-*p*-trifluoromethoxy phenyl hydrazone). Protein concentration was measured by a biuret method [7] using bovine serum albumin as a standard. The extraction of mitochondrial lipids [8] and the assay of cholesterol [9] were by standard procedures.

Hepes, ADP, succinic acid and EGTA were obtained from Sigma (London) Poole, Dorset. FCCP was from Boehringer (London) Lewes, East Sussex. All other reagents were of AR grade.

3. Results and discussion

Mature and senescent rats were allowed to survive for 18 days after partial hepatectomy to facilitate complete regeneration of the liver in senescent animals. This extended period for regeneration in the senescent animal has been attributed to a delay in the onset of DNA synthesis although thereafter, cell division and growth proceed at rates observed in the mature animal [10,11]. Mitochondria isolated from regenerated livers of senescent animals when compared with mitochondria from regenerated livers of

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Table 1
Effect of liver regeneration on respiratory control ratio

Age (months)	Liver	Respiratory control ratio	States 3 rate/ uncoupled rate	Cholesterol content of mitochondria (nmol/mg protein)
Mature (3–12)	Normal	5.86 ± 0.88* (20)	0.83 ± 0.07* (20)	10.2 ± 1.6* (11)
Old (29–33)	Normal	4.41 ± 0.34* (14)	0.62 ± 0.07* (14)	15.5 ± 1.9* (4)
Mature (3–12)	Regenerated	6.33 ± 0.66 (3)	0.84 ± 0.03 (3)	11.4 ± 1.8 (3)
Old (29–33)	Regenerated	6.36 ± 1.0 (3)	0.81 ± 0.03 (3)	13.2 ± 2.1 (3)

The number of repetitions of each experiment is indicated in brackets. Figures marked with an asterisk represent statistically significant differences by Student's t-test where $0.01 < p < 0.02$. There were no age-related differences between the maximum uncoupled rates of respiration for mitochondria isolated from control or regenerated livers

mature animals using succinate as substrate showed a complete reversal of the previously observed age-dependent decline in respiratory control ratio (RCR) and the ratio of state 3 rates of respiration to uncoupled rates (table 1). Sham operations on control animals of both age groups had no effect on the respiratory rates measured in liver mitochondria isolated 18 days after surgery. Mitochondria isolated from regenerated liver of mature animals 7 and 18 days after surgery showed no change in rates of respiration.

Hepatic regeneration also affected another age-dependent change in the inner mitochondrial membrane. Investigations of mitochondria subjected to brief exposure to hypoosmotic media had shown an age-related increase in the release of matrix proteins as measured by the activities of malate or glutamate dehydrogenase [4]. This effect was also reversed by liver regeneration. No differences were observed in the release of matrix dehydrogenases from mitochondria isolated from livers of mature rats that had undergone partial hepatectomy, sham operation or no surgery.

With regard to the mechanism of intracellular events, since hepatic regeneration, which is dependent on both nuclear and mitochondrial DNA, completely reverses these age-dependent changes in the inner mitochondrial membrane, the genetic templates used to synthesize the new cells must have remained intact, unless a previously suppressed genetic message was used. This latter suggestion is unlikely at least in the case of the mitochondrial genome which shows extreme economy in that the gene have none or only a few non-coding bases between them and in many cases even the termination codons are not coded in the DNA [12]. In the absence of further evidence that would allow a distinction to be drawn between these two possibilities, if it is assumed that the genetic templates used to synthesize the new cells had remained intact, then an explanation of the age-dependent changes may rest with the synthetic apparatus of the cell which may have become deficient as a result of stochastic damage and lower rates of turnover. Regeneration of the liver would in such a case replace the defective synthetic apparatus and restore its function completely.

Age-dependent changes examined in the outer mitochondrial membrane were unaffected by hepatic regeneration. An increase in the cholesterol content of mitochondria (mainly in the outer membrane) of as much as 30% [13] was confirmed, but regeneration of the liver in senescent animals yielded no definite change in these levels (table 1).

The suggestion that the reported reversible age-dependent effects may result from stochastic formation of modified proteins which would function less efficiently (if at all) than their intact counterparts found in mitochondria of the mature animal, is supported by the observations that modified proteins appear in senescent animals [14,15] resulting in the loss of enzymic activity. Thus post-translational modification of proteins may provide an explanation of the reversible age-dependent phenomena reported.

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