MITOCHONDRIAL HETEROGENEITY IN FOETAL AND SUCKLING RAT LIVER: DIFFERENTIAL LEUCINE INCORPORATION INTO THE PROTEINS OF TWO MITOCHONDRIAL POPULATIONS.

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SUMMARY

^{14}C-Leucine was incorporated into proteins of foetal, suckling and adult rats. Liver mitochondria were isolated and subfractionated by isopycnic gradient centrifugation. The differential labelling pattern obtained with two populations of hepatic mitochondria confirm the concept of mitochondrial heterogeneity. The results are compatible with the concept that the two populations represent alternate stages in a maturation and division cycle.

During the last fifteen years the heterogeneity of rat liver mitochondria and the separation of mitochondria into distinct and separate populations have been repeatedly investigated (1, 2, 3, 4, 5, 6, 7 and 8); the results of such studies have been criticized and questioned (9, 10, 11). Mitochondrial heterogeneity has also been reported in fractions prepared from chick liver, rabbit kidney and rat skeletal muscle (12, 13 and 14).

The separation of adult rat liver mitochondria by sucrose density gradient centrifugation into two populations (B2 and B3) (6) has been explained in terms of different accessibility of the matrix space to sucrose (6). The "leaky" (B3) mitochondria equilibrated in the denser part of the sucrose gradient and were osmotically less active than the "non-leaky" (B2) mitochondria (7). In addition it was shown with rat liver as well as chicken liver mitochondria that the ratio of B2 to B3 mitochondria was related to the developmental age of the animal (6, 12).

While these experiments showed that mitochondria may be fractionated by sucrose gradient centrifugation into two separate populations, they did not necessarily establish that two mitochondrial populations were present as such in the cell in vivo or even in the mitochondrial preparation prior to the appli-

cation to the sucrose gradient. The present study deals with the incorporation of leucine into mitochondrial proteins and is compatible with the concept that the two mitochondrial populations obtained by sucrose gradient centrifugation are not an artefact, but represent alternate stages in a maturation and division cycle.

MATERIALS AND METHODS

Animals: Rats were obtained from a random-bred Wistar strain kept at the University of Sydney Animal House, Castle Hill.

Preparation of mitochondria and isopycnic centrifugation: Foetal, suckling and adult mitochondria were isolated as described by Pollak and Munn (6). The mitochondria (2-5 mg protein in 0.2 - 0.5 ml isolation medium) were placed on a sucrose gradient as described previously (6) and centrifuged in a Spinco-Beckman SW50 rotor as described by Pollak and Morton (15). The B2 and B3 mitochondrial fractions were harvested as described before (6).

Isotope techniques: [U-^{14}C]L-leucine (specific radioactivity 10 μc/m mol) was either injected into pregnant rats 1-5 days before term, into suckling rats or into foetal rats obtained by Caesarean section. Alternatively the labeled leucine was added to 30 ml Erlenmeyer flasks which served as incubation vessels for foetal liver slices. The amount of [U-^{14}C]L-leucine used for each of the experimental conditions is indicated in Table I. After fixed time intervals (see Table I) the rats were killed and the foetal, suckling and adult livers were dissected out. Incubations of foetal rat livers *in vitro* were carried out in 15 ml Krebs-Ringer-Phosphate medium. The incubations were terminated by adding four volumes of ice-cold 0.25 M sucrose, and the slices were transferred to isolation medium. Isolated mitochondria, B2 and B3 subfractions were processed for isotope counting as described previously (16). All samples were counted in a Packard 2111 scintillation spectrometer for at least 4000 c.p.m. and corrected for counter efficiency, quenching and background.

Analytical methods: Proteins were determined by the method of Lowry et al. (17), using bovine serum albumin (Fraction V) [Sigma (St. Louis) Chemical Co. Ltd.] as standard.

RESULTS AND DISCUSSION

After ^{14}C-leucine injection or incubation, mitochondria of the B2 fraction of foetal or suckling rat liver attained a greater specific activity than the corresponding B3 fraction (Table I). This implies an inherent difference between B2 and B3 mitochondria (Table I). The difference between the specific activities of B2 and B3 mitochondria diminished as either the in vitro or as the in vivo incubation time was extended (Table IA & C).

In contrast the specific activities of B2 and B3 mitochondria obtained from similar experiments with adult rats were virtually identical (Table IE), indicating that differential leucine incorporation into B2 and B3 mitochondria depends on developmental age. It has previously also been shown that the injection of chloramphenicol into pregnant rats results in B2 and B3 mitochondria isolated from foetal liver with virtually identical specific activities (16).

The validity of the use of isopycnic sucrose centrifugation for the separation of mitochondrial populations requires some justification before the results obtained by this method can be accepted, as it has been pointed out repeatedly that rat liver mitochondria suffer damage when exposed to high sucrose concentrations (9, 10, 11 and 18). However in our hands both B2 and B3 mitochondria do exhibit some osmotic activities (7),and there are no gross differences in protein/phospholipid ratios and phospholipid composition between mitochondria prior to gradient centrifugation and B2 and B3 fractions. The specific activities of three marker enzymes for outer membrane, inner membrane and matrix of B2 and B3 mitochondria were also comparable to those of non-fractionated mitochondria.

Taking the results of sucrose density gradient centrifugation at face value, the differential incorporation of leucine into total proteins of B2 and

TABLE I

COMPARISON OF SPECIFIC ACTIVITIES OF B2 AND B3 MITOCHONDRIA AFTER ^{14}C-LEUCINE INCORPORATION

Age of rats from which liver mitochondria were isolated. Conditions of isotope labeling.	d.p.m./mg protein		B2/B3 protein ratio
	B2 mitochondria	B3 mitochondria	
A. Foetal rat liver: (3 livers/ 15 ml medium) incubated with 2μC[U-^{14}C]L-leucine corrected for 0 min label.			
5 days prenatal { 10 min Exp. 1	429	209	0.38
{ Exp. 2	617	301	0.36
{ 20 min Exp. 1	503	471	0.30
{ Exp. 2	691	563	0.34
B. Foetal rat liver: [U-^{14}C] L-leucine 4μC injected into pregnant rat, which was killed after 60 min and foetal rat liver mitochondria isolated.			
foetal age: 5 days prenatal	322	186	0.45
2 days prenatal	582	507	0.96
1.5 day prenatal	365	211	0.36
1 day prenatal	368	313	1.3
C. Caesarean delivered rats (1-day premature) injected with [U-^{14}C]L-leucine, 0.2μC			
Killed at 20 min	216	112	0.13
40 min	795	750	0.18
D. 5-6 day-old suckling rats injected with [U-^{14}C] L-leucine, 0.5μC (10mC/mmol)			
Killed after 30 min	420	372	0.8
	540	430	1.2
	310	198	2.8
E. Adult rat liver [U-^{14}C] L-leucine 4μC (from injected pregnant rats in B)	94	80	4.9
Killed after 60 min	102	118	3.6
	65	62	5.8

B3 mitochondria suggests that these two populations occur at least in foetal and suckling rat liver. As pointed out before, short periods of leucine incorporation lead to a higher labeling of B2 than B3 proteins, whereas with longer periods the specific activities of B2 and B3 approach each other. This may

MODEL OF MITOCHONDRIAL BIOSYNTHESIS, LEAKY AND NON-LEAKY MITOCHONDRIA ALTERNATING IN A CYLIC PROCESS

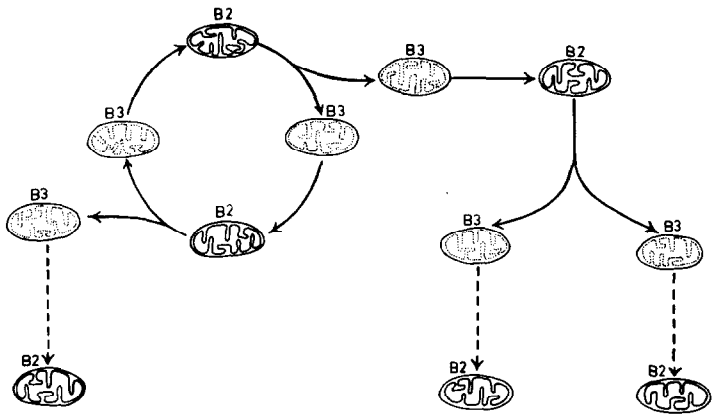

Figure 1

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be interpreted in the light of previous work (16) to be due to transformations of B2 → B3 and B3 → B2 mitochondria; B2 and B3 mitochondria representing alternate stages in a maturation and division cycle (Fig. 1). It had previously been shown that in foetal rat liver the half-lives for the total proteins were the same for both B2 and B3 mitochondria (1.68 days)(16); on the other hand the half-lives of B2 and B3 mitochondria isolated from adult rat liver were 9.6 and 4.8 days respectively. The identical half-lives of B2 and B3 mitochondria, together with the partial chloramphenicol sensitivity of amino acid incorporation of B2 (but not B3) mitochondria, as well as the 100% inhibition of amino acid incorporation by cycloheximide of B3 (but not B2) mitochondria, led to the suggestion that in B3 mitochondria no mitochondrial protein synthesis occurred, (16). The identical half-lives of total mitochondrial proteins of B2 and B3 mitochondria were interpreted to be the result of a cyclic pathway of mitochondrial biogenesis, consisting of alternating B2 and B3 generations (16 and Fig. 1). B3 mitochondria are thought to be converted by cytoplasmic protein

synthesis to B2 mitochondria and only these are considered to be able to divide to give rise to B3 mitochondria with "leaky" inner membranes (16). It is considered that in foetal rat liver B3 mitochondria predominate because cytoplasmic protein synthesis to convert B3 → B2 mitochondria is the rate-limiting step, while in adult rat liver the division of B2 mitochondria to give rise to B3 mitochondria with more permeable inner membranes is rate limiting. Thus the two mitochondrial populations represent a steady state distribution of two developmental stages.

The different labeling patterns of B2 and B3 mitochondria and the fact that increased labeling periods tended to diminish these differences support and are compatible with the concept of a cyclic process of mitochondrial biosynthesis, involving alternate steps of division and maturation of the inner membrane.

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