# ENHANCED DNA SYNTHESIS IN ISOLATED MEGAMITOCHONDRIA*

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## 1. Introduction

*Cuprizone* induced-megamitochondria in mouse liver were first described in 1969 by Suzuki [1]. These mitochondria, often exceeding the cell nucleus in diameter, are morphologically characterized by a vastly augmented matrix whereas the organization of the outer and inner membranes is essentially unchanged [2]. The phenomenon is completely reversible within approximately 8–12 hr [3] following withdrawal of the drug from the diet. Reconstitution appears to be accomplished by a division mechanism [3].

Experiments dealing with biochemical properties of these 'matrix enriched megamitochondria' [4] have not been reported so far. Characterization of biochemical parameters are, however, pertinent to an understaning of the functional significance of altered mitochondrial structure.

Biogenesis of normal mitochondria depends primarily on an intact DNA synthesizing system as shown by several investigators [5–7]. In order to examine whether formation of giant mitochondria implies an altered DNA synthesis the kinetics of [$^3$H]thymidine incorporation by isolated mitochondria were determined. The newly synthesized DNA was characterized on cesium chloride–ethidium bromide gradients. It will be demonstrated: i) that megamitochondria exhibit a different DNA synthesis activity; ii) the DNA content of megamitochondria is significantly altered; iii) determination of mitochondria-associated acid-soluble radioactivity under the experimental conditions applied suggested that nucleotide pool alterations were not involved.

## 2. Materials and methods

Weanling male mice (NMRI strain) weighing approximately 10 g were placed on a powdered complete diet containing 0.6% *Cuprizone* (biscyclohexanone oxaldihydrazone). After 10–12 days depending on the clinical appearance mice were killed and the livers removed under sterile conditions as described by Beattie et al. [8]. The livers were homogenized in 20 vol of isolation medium consisting of 0.25 M sucrose, 10 mM HEPES pH 7.4, 2 mM EDTA (sodium salt) and 30 mM nicotinamide by means of a Dounce type homogenizer with a loose fitting pestle. The homogenate was centrifuged at 560 *g* for 15 min. The supernatant was centrifuged at 7000 *g* for 15 min and the pellet discarded. The fluffy layer was carefully removed, and the pellet washed twice after gentle resuspension in one-half and one-fourth of the original volume of isolation medium followed by centrifugation at 7000 *g* for 15 min. Examination of isolated normal and giant mitochondria at the ultrastructural level revealed an essentially intact morphology of the organelles (manuscript in preparation).

*In vitro* labelling of isolated mitochondria was carried out according to the method of Mitra and Bernstein [9] with minor modifications. The incuba-

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tion assay contained: 0.5 μM [$^3$H]thymidine (specific activity 20 Ci/mmol), 0.05 mM dATP, 0.05 mM GTP, 0.05 mM dCTP, 3 mM ATP, 10 mM succinate, 2 mM pyruvate, 1 mM malate, 2 mM nicotinic acid (potassium salt), 10 mM $MgCl_2$, 2 mM KCl, 10 mM $KH_2PO_4$, 30 mM Bicine pH 8.0, 20 μg DNAase per ml and 200 IU penicillin per ml. Incubation was performed in a metabolic shaker at 36°C with a final concentration of 1 mg mitochondrial protein per ml. Bacterial contamination could be excluded by plating aliquots of the incubation assay (after 1 hr of incubation) on blood agar.

After several washings of the labelled mitochondria with isolation medium in order to remove DNAase, mitochondrial DNA was extracted following essentially the method described by Hirt [10] and characterized by isopycnic centrifugation in cesium chloride–ethidium bromide solution [11]. DNA was determined by the method of Giles and Myers [12] as described by Kimberg and Loeb [13]. Protein was estimated by the method of Lowry et al. [14]. Determination of radioactivity was performed as reported previously [15].

Table 1

DNA content, specific radioactivity of mitochondrial DNA and acid-soluble radioactivity of isolated mitochondria from normal mouse liver and livers from *cuprizone* treated animals labelled with [$^3$H]thymidine *in vitro*.

| | DNA content (μg DNA/mg mitochondrial protein) | Specific radioactivity of mitochondrial DNA (cpm/μg DNA) | Acid-soluble radioactivity (cpm/mg mitochondrial protein) |
|---|---|---|---|
| Control | 0.56 | 5082 | 1186 |
| *Cuprizone* induced megamitochondria | 0.36 | 11 211 | 978 |

## 3. Results

As shown in fig. 1 the kinetics of [$^3$H]thymidine incorporation into isolated liver mitochondria of *cuprizone* treated mice exceeded that of the controls at least 2-fold. This enhancement was not due to an increased DNA content as demonstrated in table 1. In contrast, *cuprizone* induced-megamitochondria exhibited a significantly lower DNA/protein ratio.

These findings needed further support by examination of the specific radioactivity of mitochondrial DNA. Labelling of isolated mitochondria for 1 hr was followed

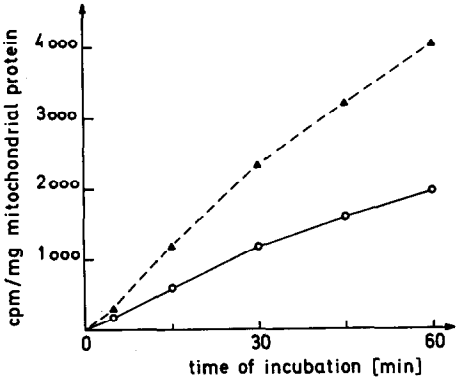


Fig. 1. Incorporation of [$^3$H]thymidine by isolated liver mitochondria from untreated (o—o—o) and *cuprizone* treated animals (▲---▲---▲). Mitochondria were isolated after 10–12 days of treatment. *Cuprizone* induced giant liver mitochondria exhibit a higher incorporating activity than that of the controls.

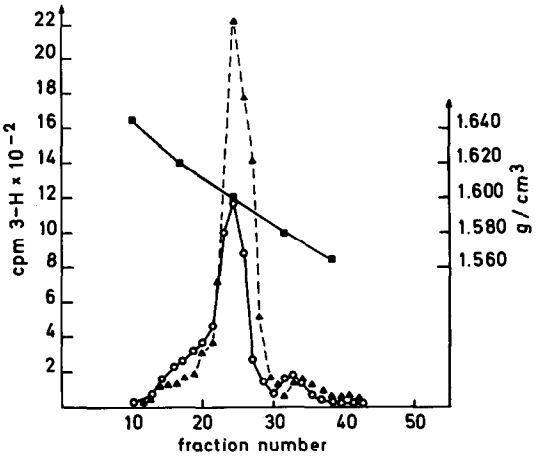


Fig. 2. Isopycnic centrifugation in cesium chloride–ethidium bromide solution of DNA extracted from isolated mitochondria of mouse liver after *in vitro* labelling with [$^3$H]thymidine for 1 hr (o—o—o) and DNA extracted from isolated *cuprizone* induced-giant liver mitochondria labelled under identical conditions (▲---▲---▲). The radioactivity in the position of the covalently closed double stranded circular DNA of *cuprizone* induced-giant mitochondria exceeds that of the controls significantly.

by determination of acid-precipitable radioactivity and DNA content. Evaluation of the specific radioactivities of DNA from giant mitochondria and that of the controls (table 1) confirmed the results of [$^3$H]-thymidine incorporation (fig. 1) and DNA content (table 1) as described above.

Analysis of the radioactive products from equal amounts of mitochondrial protein by isopycnic centrifugation in cesium chloride–ethidium bromide gradients revealed essentially similar patterns. The covalently closed double stranded circular DNA of both samples could be recovered at a density of 1.6 g/m$^3$ whereas a minor component banded at a density of approximately 1.57 g/cm$^3$ corresponding to linear DNA fragments. Pipetting of the mitochondrial DNA prior to isopycnic centrifugation caused a significant loss of radioactivity in the position of the circular double stranded DNA and a corresponding increase in the position of the linear double stranded DNA.

The amount of radioactivity, however, recovered from gradients that had been loaded with mitochondrial DNA from megamitochondria exceeded that of the controls significantly (fig. 2). This result had to be expected considering the differences in specific radioactivity and DNA content.

## 4. Discussion

Our results demonstrate that isolated *cuprizone* induced-megamitochondria exhibit an enhanced DNA synthesis activity as compared to controls. It could be established that an increased or an altered intramitochondrial nucleotide pool were not involved. For an interpretation of these results it has to be pointed out that our experiments were performed on isolated organelles, i.e. after removal of megamitochondria from the intracellular environment.

Morphological studies on megamitochondria reported recently [2] and revealed a decreased ratio between the inner mitochondrial membrane and the matrix. These structural alterations are essentially comparable to those induced by ethidium bromide [16, 17], a potent inhibitor of mitochondrial nucleic acid and protein synthesis. Assuming an analogy between *cuprizone* action and ethidium bromide effect an inhibition of mitochondrial protein synthesis as part of the *cuprizone* intoxication has to be considered. As our results were obtained from isolated organelles one can only speculate that the *in vitro* system might simulate the enzymatic activities preceding mitochondrial division during the recovery phase. This hypothesis has to be supported by determination of mitochondrial synthesizing activities *in vivo* during formation of megamitochondria and recovery phase. Investigations on this line are in progress.

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## References

[1] Suzuki, K. (1969) Science 163, 81.
[2] Albring, M. and Thoenes, W. (1973) Verhandl. Deut. Ges. Pathol. 57, in press.
[3] Tandler, B. and Hoppel, C.L. (1973) J. Cell Biol. 56, 266.
[4] Thoenes, W. (1966) Z. Zellforsch. 75, 422.
[5] Coote, J.L. and Work, T.S. (1971) European J. Biochem. 23, 564.
[6] Borst, P. (1972) Ann. Rev. Biochem. 41, 792.
[7] Kroon, A.M., Agsteribbe, E. and deVries, H. (1972) in: The Mechanism of Protein Synthesis and its Regulation (Bosch, L., ed.) p. 539, North-Holland, Amsterdam.
[8] Beattie, D.S., Basford, R.E. and Koritz, S.B. (1967) J. Biol. Chem. 242, 3366.
[9] Mitra, R.S. and Bernstein, B.A. (1970) J. Biol. Chem. 245, 1255.
[10] Hirt, B.J. (1967) J. Mol. Biol. 26, 365.
[11] Radloff, R., Bauer, W. and Vinograd, J. (1967) Proc. Natl. Acad. Sci. U.S. 57, 1514.
[12] Giles, K.W. and Myers, A. (1965) Nature 206, 93.
[13] Kimberg, D.V. and Loeb, J.N. (1971) Biochim. Biophys. Acta 246. 412.
[14] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1953) J. Biol. Chem. 193, 265.
[15] Radsak, K., Kato, K., Sato, N. and Koprowski, H. (1971) Exptl. Cell Res. 66, 410.
[16] Soslau, G. and Nass, M.M.K. (1971) J. Cell Biol. 51, 514.
[17] Nass, M.M.K. (1972) Exptl. Cell Res. 72, 211.