

case of several histocompatibility *loci*, provided that selective elimination of homozygotes of each allele is greater than elimination of all heterozygotes involving that allele, and that females discriminate against offspring of their own genotype (Hull, 1966).

It would appear that none of these mechanisms suggested by Snell can be discounted completely on theoretical grounds as being responsible for the mixture of histocompatibility genotypes which is inferred to exist in mammalian populations. More data on the actual distribution of histocompatibility type between and within families would be necessary to decide between these hypotheses.

### 3. SUMMARY

Of the mechanisms suggested by Snell as being responsible for the maintenance of histocompatibility differences in mammalian populations, none of those which was examined theoretically could be discounted completely as a possible cause.

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## HETEROGENEITY OF MITOCHONDRIA IN THE INTERSPECIFIC HYBRID *MESOCRICETUS NEWTONI* × *M. AURATUS*

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### 1. INTRODUCTION

THE occurrence of mixed mitochondrial populations in the cells, demonstrated cytochemically for the succinate-dehydrogenase and cytochrome-oxidase in "petites" strains of *Saccharomyces* (Avers *et al.*, 1965) raises the question of the complexity of the ways by which the uniformity of the cytoplasmic determinants is accomplished, either following the production of mutated genetic factors within the intracellular mitochondrial population, or following the combination by hybridisation of two different mitochondrial populations.

Another remarkable example of mitochondrial heterogeneity has been described in maize (McDaniel and Sarkissian, 1966, 1968), which could be

explained assuming the gametic transmission of the two types of parental mitochondria.

The present paper is based upon a comparison between the behaviour of the liver mitochondria from the interspecific hybrid *Mesocricetus newtoni* × *M. auratus* during aging of mitochondrial preparation, with that of an *in vitro* mixture of mitochondria from the two parental species.

The interspecific hybrid *M. newtoni* × *M. auratus* was described and cytogenetical and histological studies were reported by Raicu and Bratosin (1968) and Raicu *et al.* (1969). The study of mitochondrial ageing was concerned with three parameters: mitochondrial swelling, DNP-induced mitochondrial ATP-ase and release of intramitochondrial magnesium, as Chefurka (1966) indicates differences between species for the kinetics of mitochondrial ageing for these parameters.

## 2. MATERIALS AND METHODS

Males of *M. newtoni* and *M. auratus* from the Department of Genetics (University of Bucharest) were used in this study, as well as male individuals from the interspecific hybrid obtained in this department.

Liver mitochondria were prepared by the procedure of Hogeboom (1955), and *in vitro* 1:1 mixtures of mitochondria were made by mixing aliquots of the supernatants resulting from the first centrifugation (at 3000 g), and centrifuging the mixture in the usual manner for the sedimentation of mitochondria.

Ageing of mitochondria was studied in 0.25 M sucrose at 35° C. Samples were removed at specified times and analysed for the three ageing parameters. Mitochondrial swelling was studied following the decrease in optical density in 0.25 M sucrose at 520 m $\mu$  in a Beckman DU spectrophotometer, using quartz cuvettes of 1 cm. The mitochondrial suspension was adjusted with 0.25 M sucrose to contain 1.2 mg. protein per sample.

ATP-ase activity was studied in the following reaction mixture: 75 mM KCl, 1 mM EDTA pH 7.2, 50 mM Tris-acetate buffer pH 7.2, 50 mM sucrose, 2 mM ATP di-Na salt pH 7.2. For the study of DNP-induced ATP-ase, 0.33 mM DNP was added to the reaction mixture. Final volume was 1.5 ml., including the mitochondrial suspension (about 1.5 mg. protein). After incubation for 15 minutes at 25° C., the reaction was stopped with 0.1 ml. PCA 7 per cent. Inorganic Phosphorus was determined by the Lowry and Lopez method (Leloir and Cardini, 1957). Enzyme activity was expressed as  $\mu$  moles  $P_i$  formed in 15 minutes by 1 mg. protein in the above conditions.

The release of intramitochondrial  $Mg^{++}$  was studied after Chefurka (1966) and magnesium was determined fluorimetrically by the method of Schachter (1959). Protein was determined with Folin-Ciocalteu reagent (Layne, 1957). All the determinations were made at least in triplicate.

## 3. RESULTS AND DISCUSSION

The curves of the three parameters studied during ageing of the mitochondrial preparation at 35° C. show striking differences between the two species. Fig. 1 shows the evolution of the mitochondrial swelling, during 25 minutes of ageing at 35° C., for liver mitochondria from *M. newtoni*, *M. auratus*, the interspecific hybrid and the mixture of parental mitochondria.

It can be seen that the optical density of the mitochondria from the hybrid organism is intermediate between those of the two genitors. Furthermore, a striking identity appears between the curve of swelling of the hybrid and that of the *in vitro* mixture.

Fig. 2 shows the decrease of DNP-induced ATP-ase activity at pH 7.2, which seems to be a characteristic parameter of mitochondrial ageing (Chefurka, 1966). The low ATP-ase activity in the absence of DNP and its small increase during ageing proves the good state of the mitochondrial preparations and reveals the differential activity induced activity by DNP.

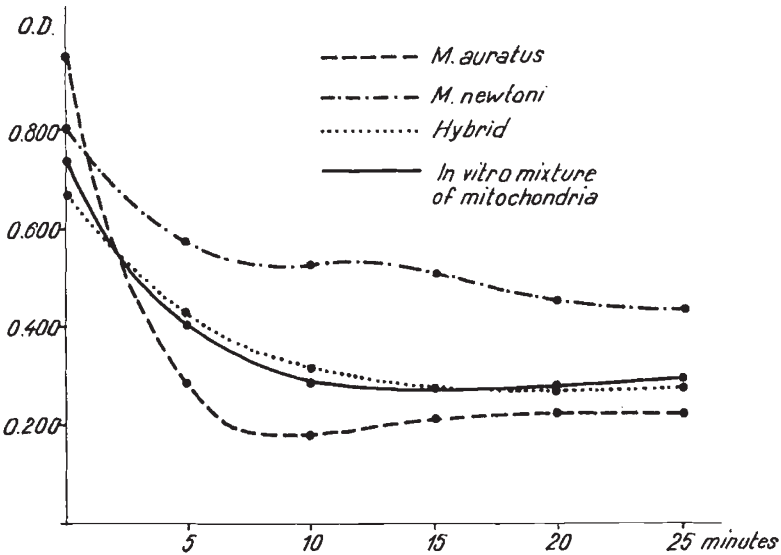


FIG. 1.—Mitochondrial swelling during ageing.

As in the case of mitochondrial swelling, the evolution of the DNP-induced ATP-ase during ageing reveals the intermediate properties of the mitochondria from the hybrid and their quasi-identity with the *in vitro* mixture of mitochondria from genitors.

The same can be seen from the analysis of the release of intramitochondrial magnesium (fig. 3): the rates of loss of intramitochondrial magnesium for the mitochondria of the hybrid are intermediate between that of the genitors, and nearly identical with the rates of magnesium loss from the mitochondria in the *in vitro* mixture.

The striking similarities observed between the behaviour of the mitochondria of the hybrid organism and that of the mitochondria in the artificial mixture of mitochondria, as far as the three parameters studied are considered, suggest the occurrence of mixed mitochondrial populations in the cells of the hybrid organism.

It should be noted that all the three parameters studied are concerned in phenomena which are directly or indirectly related to the properties of mitochondrial membranes and it seems proved (Munkres, 1968; Munkres and Woodward, 1967; Woodward and Munkres, 1966, 1967) that the synthesis and the properties of mitochondrial structural protein are under the control of the mitochondrial genetic apparatus.

Like the other evidence for the occurrence of mixed mitochondrial population (Avers *et al.*, 1965; McDaniel and Sarkissian, 1966, 1968) the heterogeneity of mitochondria of the interspecific hybrid *M. newtoni* × *M.*

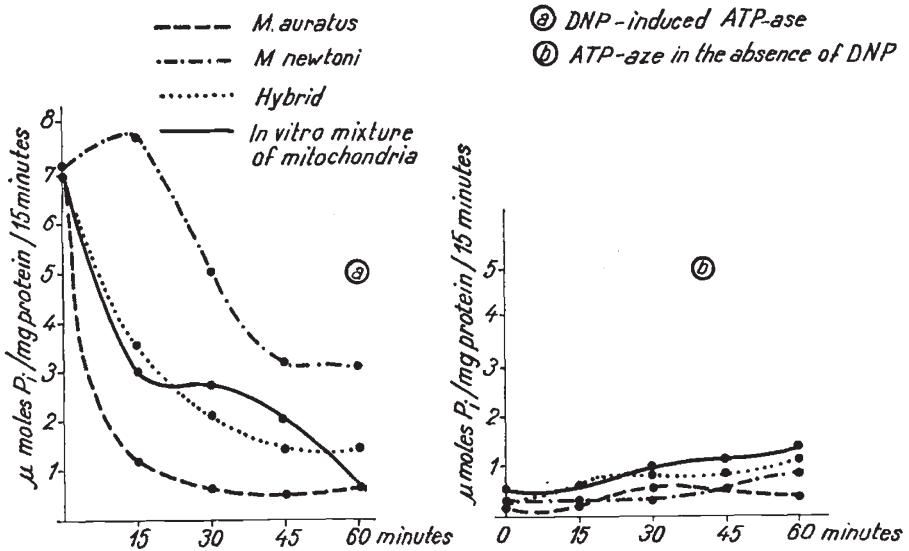


FIG. 2.—Decrease of DNP-induced ATP-ase activity during ageing of mitochondrial preparations.

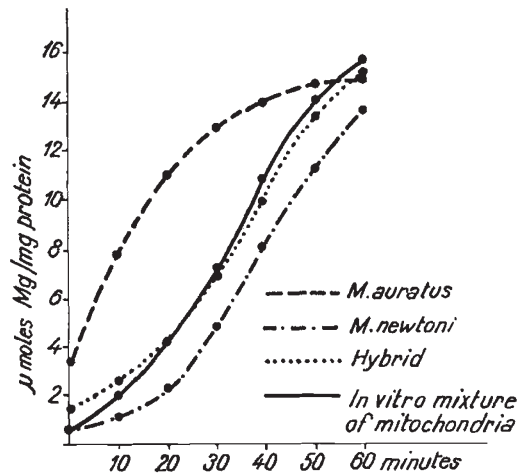


FIG. 3.—Release of intramitochondrial magnesium during ageing.

*auratus* indicates the possibility that different mitochondria may produce "heterozygous" combinations, like different allelic genes, resulting in a condition similar to heterokaryosis or nuclear heterogeneity in fungi (Wagner, 1969).

## 4. SUMMARY

1. Ageing of liver mitochondria from the interspecific hybrid *M. newtoni* × *M. auratus* was studied for three parameters (mitochondrial swelling, DNP-induced ATP-ase, release of intramitochondrial magnesium), as compared both to the two genitor species and to an *in vitro* mixture of mitochondria from the two parental species.

2. A nearly perfect identity was found between the behaviour of the mitochondria from the hybrid organism and that of the mitochondria in the mixture.

3. The results suggest the occurrence of mixed mitochondrial populations in the cells of hybrid organisms.

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