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Aging Changes of Macromolecular Synthesis in the Mitochondria of Mouse Hepatocytes as Revealed by Microscopic Radioautography

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Abstract

Nagata T. Aging Changes of Macromolecular Synthesis in the Mitochondria of Mouse Hepatocytes as Revealed by Microscopic Radioautography. ARBS Annu Rev Biomed Sci 2007;9:30-36. This mini-review reports aging changes of macromolecular synthesis in the mitochondria of mouse hepatocytes. We have observed the macromolecular synthesis, such as DNA, RNA and proteins, in the mitochondria of various mammalian cells by means of electron microscopic radioautography technique developed in our laboratory. The number of mitochondria per cell, number of labeled mitochondria per cell with ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-leucine, precursors for DNA, RNA and proteins, respectively, were counted and the labeling indices at various ages, from fetal to postnatal early days and several months to 1 and 2 years in senescence, were calculated, which showed variations due to aging.

Keywords: radioautography, DNA, RNA, proteins, liver, mouse, aging

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1. Introduction

Intramitochondrial nucleic acid syntheses, both DNA and RNA, in mammalian and avian cells were first demonstrated morphologically by the present author by means of electron microscopic radioautography with accurate localization in primary cultured cells of the livers and kidneys of mice and chickens in vitro (Nagata *et al*., 1967, 1979, 1982), and then in some other established cell lines such as HeLa cells (Nagata, 1972a,b,c) or mitochondrial fractions prepared from *in vivo* cells (Nagata, 1974; Nagata *et al*., 1975). It was later commonly reported in various cells and tissues, not only *in vitro* obtained from various organs *in vivo* (Nagata, 1974; Nagata & Murata, 1977; Nagata *et al*., 1977) but also *in vivo* cells of various organs as previously reviewed in monographs (Nagata, 2001, 2002).

2. Techniques of Radioautography

We have developed techniques of microscopic radioautography for both light and electron microscopy (Nagata, 1992, 1996, 1997, 2002). For this study, the liver tissues were obtained from 30 groups of normal ddY strain mice, each consisting of 3 litter mates of both sexes, total 90, aged from embryonic day 19 to postnatal days 1, 3, 9 and 14, and months 1, 2, 6, 12, and year 1 and 2 were used. They were administered with ^{3}H-thymidine, ^{3}H-uridine or ^{3}H-leucine, DNA, RNA and protein precursors, respectively, and the tissues were processed for light and electron microscopic radioautography.

3. Mitochondrial Macromolecular Synthesis

3.1. Mitochondrial DNA synthesis in the liver

We studied the liver tissues of ddY strain mice at various ages from embryonic day 19 to postnatal 2 years (Ma *et al*., 1994, Ma & Nagata, 1988; Nagata, 2007a,b,c). Observing light microscopic radioautograms labeled with ^{3}H-thymidine, the silver grains were found over the nuclei of some hepatocytes in S-phase, demonstrating DNA synthesis. By electron microscopic radioautography, we observed some nuclei and some mitochondria in hepatocytes in perinatal stages at embryonic day 19, postnatal day 1 (Fig. 1A), 3, 9 and day 14 (Fig. 1B) as well as young adult and senescent animals. However, those labeled hepatocytes were almost mononucleate cells and only a few binucleate cells were found among the mononucleate hepatocytes (Nagata & Ma, 2003). On the other hand, labeled binucleate hepatocytes over their nuclei were found only at the perinatal stages from postnatal day 1 to 14, but not after postnatal month 1 to senescent stages up to month 24. Among many unlabeled hepatocytes in the nuclei, both mononucleate and binucleate, several silver grains were observed over their mitochondria due to the incorporations of ^{3}H-thymidine especially at the perinatal stages from embryonic day 19 to postnatal day 14 (Fig. 1B). The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices but some grains were over the mitochondrial membranes and cristae when observed by high power magnification (Fig. 1B). The number of mitochondria and the labeling indices were calculated in both mononucleate and binucleate hepatocytes with labeled or unlabeled nuclei at respective aging stages. The results are in Fig. 1C (mononucleate) and Fig. 1D (binucleate). The number of mitochondria in mononucleate hepatocytes increased from the prenatal day to postnatal day 14, reached the maximum at the postnatal month 1-6, and then decreased to year 1-2. Thus, the number of mitochondria labeled with silver grains obtained from each animal in 7 aging groups were plotted and the labeling indices in respective aging stages were calculated. As for the binucleate hepatocytes, on the other hand, the number of mitochondria in binucleate hepatocytes (Fig. 1D), the number of labeled mitochondria per binucleate cell and the labeling index in 4 groups from postnatal day 1 to 14, stayed unchanged. The total numbers of labeled and of unlabeled mitochondria as well as the number of labeled mitochondria were more in binucleate than in mononucleate cells.

3.2. Mitochondrial RNA synthesis in the liver

Observing light microscopic radioautograms labeled with ^{3}H-uridine, the silver grains were found over both the karyoplasm and cytoplasm of almost all cells not only at the perinatal stages from embryo day 19 to postnatal day 1-14, but also at the adult and senescent stages from postnatal month 1 to 24. By electron microscopic observation, silver grains were observed in most mononucleate hepatocytes in respective aging groups localizing not only over euchromatin and nucleoli in the nuclei but also over many other cell organelles, such as endoplasmic reticulum, ribosomes and

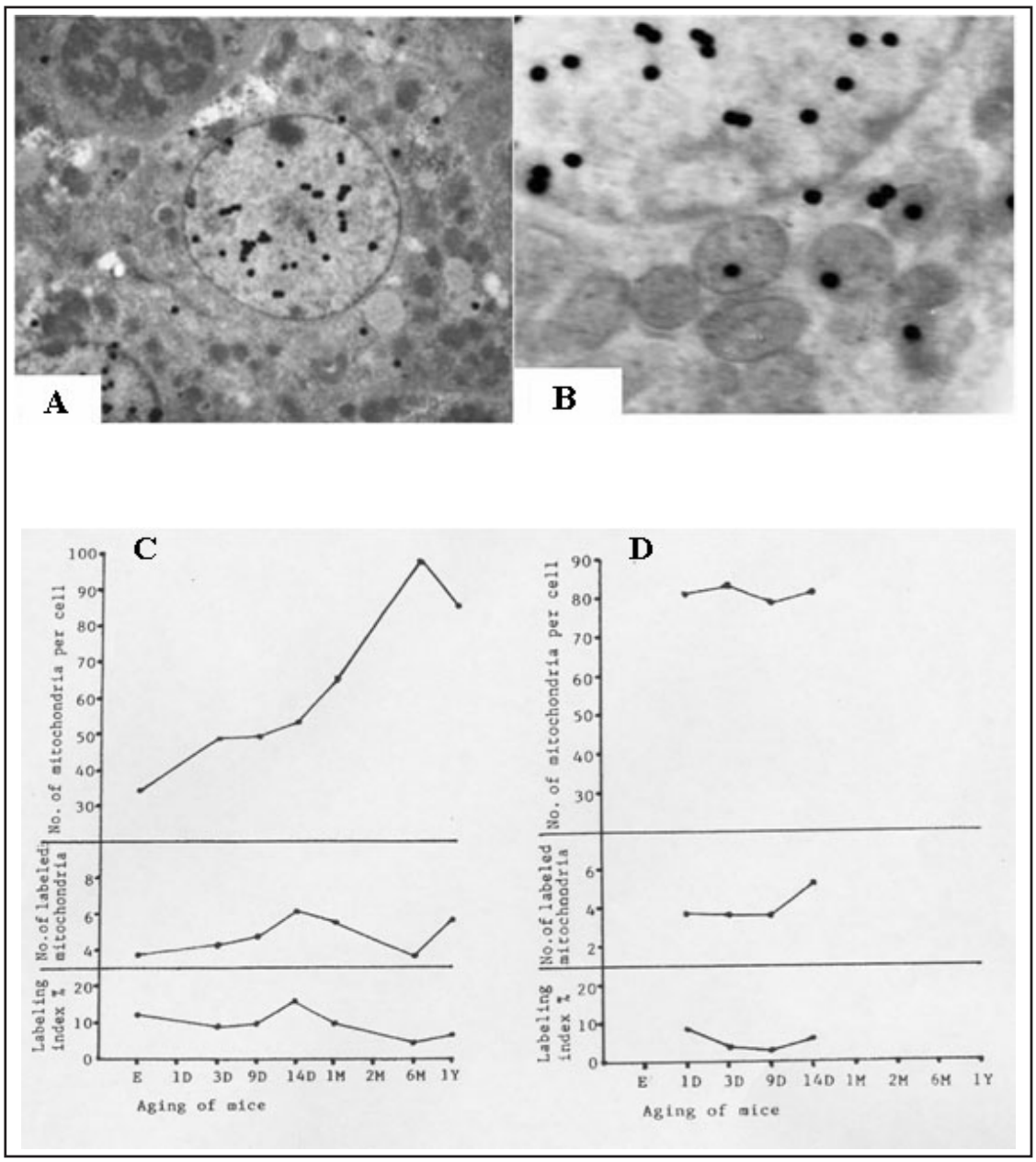

Figure 1. A, B: Electron microscopic radioautograms of the livers of newborn mice at postnatal day 1 (A) and day 14 (B) labeled with ^{3}H-thymidine, demonstrating DNA synthesis in the nuclei and mitochondria. Magnification x3,000 (A) and x15,000 (B). C, D: Transitional curves demonstrating aging changes of mitochondria in mononucleate (C) and binucleate (D) hepatocytes labeled with ^{3}H-thymidine, mean ± standard deviations; top, plotted averages of the total number of mitochondria per cell in mononucleate and binucleate hepatocytes at respective aging groups from embryonic day 19 to postnatal year 2; middle, plotted averages of the total number of mitochondria labeled with ^{3}H-thymidine indicating DNA synthesis per cell in mononucleate and binucleate hepatocytes at the respective aging groups; bottom, plotted averages of the labeling index of mitochondria labeled with ^{3}H-thymidine evidencing DNA synthesis (number of labeled mitochondria/number of total mitochondria) at respective aging groups.

mitochondria, as well as cytoplasmic matrices from perinatal stage at embryonic day 19, postnatal day 1, 3 (Fig. 2A), 9 and 14 (Fig. 2B), to adult and senescent stages at postnatal month 1-24 (Nagata & Ma, 2005a,b; Nagata, 2007a). The silver grains were also observed in binucleate hepatocytes at postnatal day 1 to month 24. The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices, but a few occurred over the mitochondrial membranes and cristae when observed by high power magnification (Fig. 2B). Moreover, almost all the hepatocytes were labeled with silver grains indicating RNA synthesis in their nuclei and mitochondria (Nagata & Ma, 2005a,b). The number of mitochondria and the labeling indices were calculated for each animal

in respective aging stages. The results showed that the number of mitochondria in mononucleate increased from the prenatal day to postnatal month 1-2, reaching the maximum, and then decreased to year 1-2. The number of mitochondria labeled with silver grains and the labeling indices in respective aging stages increased from postnatal day 1 to postnatal day 9, remained almost constant from postnatal day 9 to month 1 and decreased to month 2-6, and slightly increased to year 1-2. As

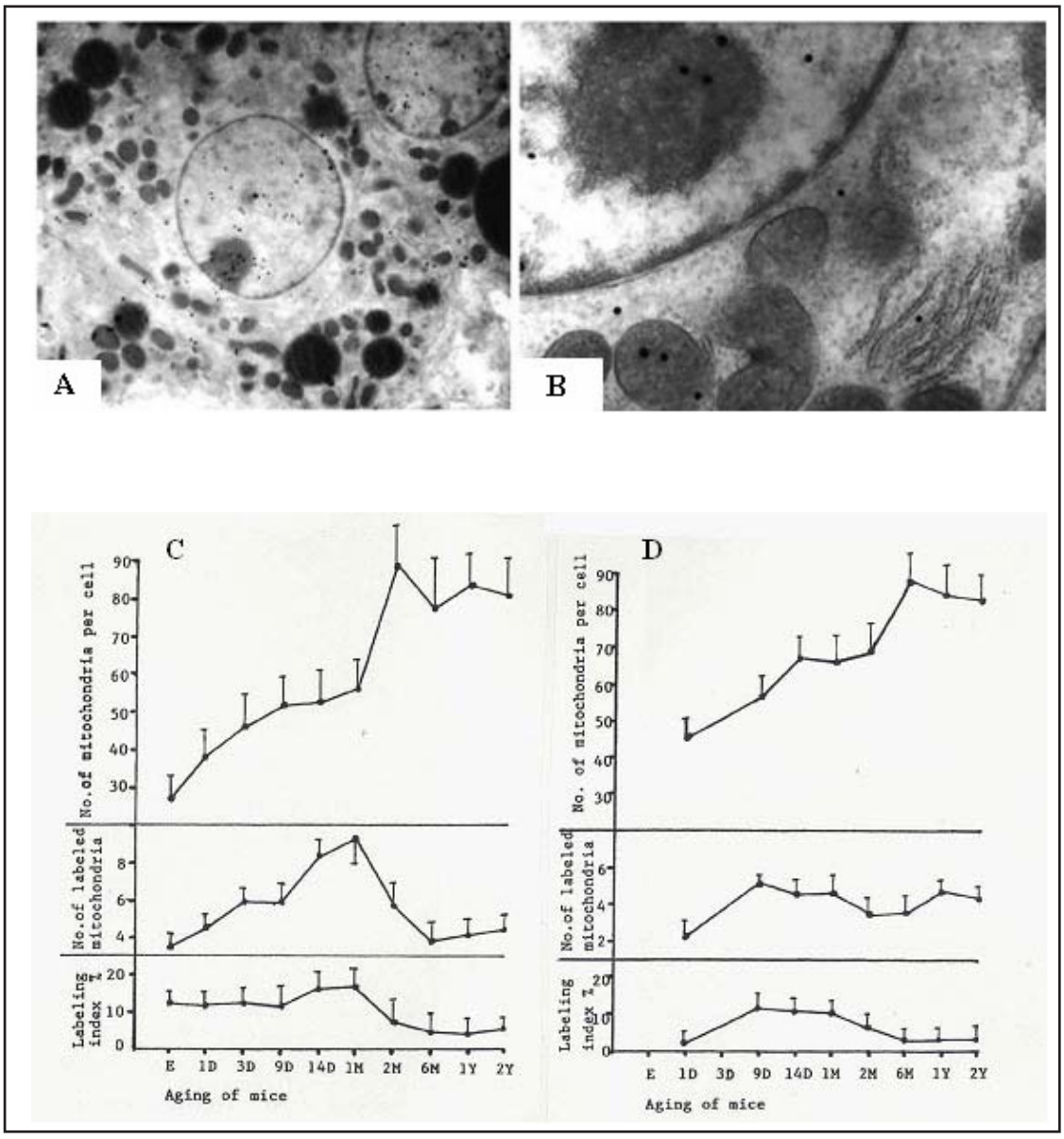

Figure 2. A, B: Electron microscopic radioautograms of the livers of newborn mice at postnatal day 3 (A) and 14 (B), labeled with ^{3}H-uridine, demonstrating RNA synthesis in the nuclei and mitochondria. Magnification: x3,000 (A) and x15,000 (B). C, D: Transitional curves demonstrating aging changes of mitochondria in mononucleate (C) and binucleate (D) hepatocytes labeled with ^{3}H-uridine, mean ± standard deviations; *a*top, plotted averages of the total number of mitochondria per cellular profile area in mononucleate and binucleate hepatocytes at respective aging groups from embryonic day 19 to postnatal year 2; middle, plotted averages of the total number of mitochondria labeled with ^{3}H-uridine indicating RNA synthesis per cellular profile area in mononucleate and binucleate hepatocytes at respective aging groups; bottom, plotted averages of the labeling index of mitochondria labeled with ^{3}H-uridine showing RNA synthesis (number of labeled mitochondria / number of total mitochondria) at respective aging groups.

for the binucleate hepatocytes, the number of labeled mitochondria increased from postnatal day 1 to day 9, and remained almost constant to year 2, but the labeling indices increased from postnatal day 1 to postnatal day 9, remained almost constant from postnatal day 9 to month 1, then decreased to month 2-6 and slightly increased to year 1-2 (Fig. 2D).

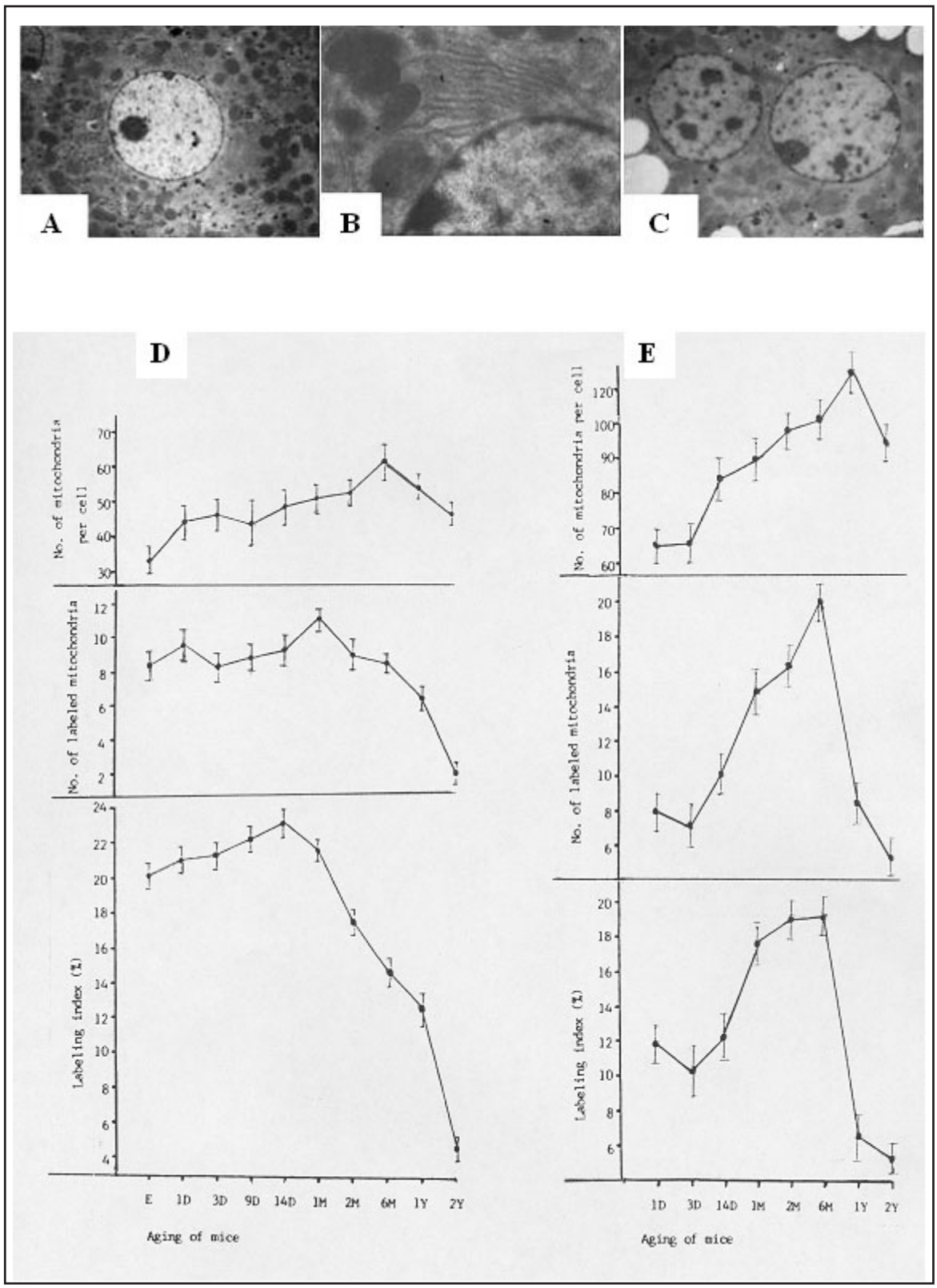

Figure 3. Electron microscopic radioautograms of the livers of newborn mice at postnatal day 1 (A) and 14 (B) and an adult at month 2 (C), labeled with ^{3}H-leucine, evidencing protein synthesis of mononucleate (AB) and binucleate (C) hepatocytes. D, E: Transitional curves indicating aging changes of mitochondria in mononucleate (D) and *d*binucleate (E) hepatocytes labeled with ^{3}H-leucine, mean ± standard deviations; top, plotted averages of the total number of mitochondria per cellular profile area in mononucleate and binucleate hepatocytes at respective aging groups from embryonic day 19 to postnatal year 2; middle, plotted averages of the total number of mitochondria labeled with ^{3}H-leucine showing RNA synthesis per cellular profile area in mononucleate and binucleate hepatocytes at respective aging groups; bottom, plotted averages of the labeling index of mitochondria labeled with ^{3}H-leucine showing protein synthesis (number of labeled mitochondria / number of total mitochondria) at respective aging groups.

3.3. Mitochondrial protein synthesis in the liver

When the animals were injected with ^{3}H-leucine, almost all the mononucleate hepatocytes, from embryonic day 19, postnatal day 1, 3, 9 (Fig. 3A) and 14 (Fig. 3B) to adult and senescent stages at postnatal month 1-24, were labeled (Nagata, 2006b, 2007c). The silver grains were also observed in binucleate hepatocytes at postnatal day 1 to month 1, 2 (Fig. 3C), 6 , 12 and 24. Silver grains observed over the mitochondria were located mainly on the mitochondrial matrices, but a few over their nuclei, cytoplasmic matrix, endoplasmic reticulum, ribosomes, Golgi apparatus and mitochondria (Nagata, 2006a,b, 2007c). In the mitochondria, the silver grains were over the mitochondrial membranes and cristae when observed by high power magnification (Fig. 3B). The numbers of mitochondria and of labeled mitochondria and the labeling indices were calculated. The total numbers of mitochondria in mononucleate hepatocytes increased from the prenatal day to postnatal days 1-14, to postnatal months 1-2, reaching the maximum at month 6, then decreased to years 1 and 2 (Fig. 3D). The numbers of mitochondria labeled with silver grains were labeled with ^{3}H-leucine, thus indicating protein synthesis in each group at perinatal stages to postnatal day 1 to year 1-2 (Fig. 3D). The labeling indices in respective aging stages (Fig. 3E) showed that the numbers of labeled mitochondria increased from prenatal embryo day 19 to postnatal month 1, reaching the maximum, and then decreased to month 2-year 2, while the labeling indices increased from prenatal day 19 to postnatal day 1-14 , reaching the maximum, then decreasing to month 1 to year 1-2. The increases and decreases of the numbers of labeled mitochondria, as well as the labeling indices from the perinatal stage to the adult and senescent stages, were statistically significant.

3.4. Mitochondrial macromolecular synthesis in the other organs

We have also studied on the mitochondria of the other organs such as the adrenal glands (Nagata, 2008), the lungs and the testis of aging mice, which also changed similarly to the hepatocytes. However, the timings of the increases or decreases were a little different from the respective organs suggesting organ specificity. Thus, we recommend future studies of such aging changes in all the important organs of non-human and human bodies.

4. Concluding Remarks

From the results reported, nucleic acids, both DNA and RNA, and protein syntheses showing incorporations of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-leucine were demonstrated in the nuclei and mitochondria of hepatocytes of the livers of aging mice at various ages from fetal to postnatal newborn, juvenile, young, adult and senescence. The numbers of mitochondria per cell, the numbers of labeled mitochondria and the labeling indices of hepatocytes increased and decreased, irrespective of nuclei. These results indicate that the mitochondria in respective cell types of these organs synthesize DNA, RNA and proteins by themselves, increase and decrease their numbers per cell with aging of the individual animals and proliferate and metabolize ion independently from the proliferations of their host cells.

We have also studied the mitochondria of other organs (e. g., adrenal glands, lungs and testis) of aging mice, which also showed variations similarly to the hepatocytes. However, the timings of the increases or decreases were a little different from respective organs, thus reflecting organ specificity. These studies are now still in progress. Therefore, it seems very important to analyze further such aging changes in all the important organs of non-human and human bodies in the future.

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