Electron Microscopic Radioautography and Its Application to DNA, RNA and Protein Synthesis in Mitochondria of Various Animal Organs

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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 in 1967 and then in some other established human cell lines such as HeLa in 1972 or mitochondrial fractions prepared from in vivo cells from mice and rats. It was later commonly found in various cells and tissues not only in vitro but also in vivo cells of various organs such as the salivary glands, the liver, the pancreas, the trachea, the lung, the kidney, the testis, the uterus, the adrenal gland, the brain, the retina of chickens, mice and rats. The relationship between the intramitochondrial DNA synthesis and cell cycle was formerly studied and it was clarified that the intramitochondrial DNA synthesis was performed without any nuclear involvement. However, the relationship between the DNA synthesis and the aging of individual animals has not yet been fully clarified. Lately, other macromolecules such as RNA and proteins were also demonstrated that they were synthesized in mitochondria of various organs not only in the nucleus, nuclei or endoplasmic reticulum. This paper deals with the relationships between the DNA, RNA and protein syntheses in the adrenal cells, the pulmonary cells and the testicular cells of mice in vivo at various developmental and aging stages to senescence from fetal day 19 to postnatal 2 years as observed by electron microscopic radioautography.

Keywords: mitochondria, DNA, RNA and protein synthesis, radioautography, adrenal gland, lung, testis, mice

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 [1] and then in some other established cell lines such as HeLa cells [2-4] or mitochondrial fractions prepared from in vivo cells from mice [5,6]. It was later commonly found in various cells and tissues not only in vitro obtained from various animal organs [7, 8, 9] but also in vivo cells of various organs such as the salivary glands [10], the liver [11-17], the pancreas [18, 19], the trachea [20], the lung [21, 22], the kidney [23], the testis [24-26], the uterus [27, 28], the adrenal gland [29, 30], the brain [31], the cornea and retina [32-35] of chickens, mice and rats. The relationship between the intramitochondrial DNA synthesis and cell cycle was formerly studied and it was clarified that the intramitochondrial DNA synthesis was performed without any nuclear involvement [36-38]. This paper deals with the relationship between the DNA, RNA and protein syntheses and the developments in several organs such as hepatocytes in the liver, adrenal cells, pulmonary cells and testicular cells of ddY strain mice in vivo at various developmental stages from fetal day 19 to postnatal month 24 by means of electron microscopic radioautography.

2. Techniques of Radioautography

2.1 Animals

In general, small experimental animals such as mice and rats should be used, because of the minimal quantities of the RI-labeled macromolecular precursors needed for radioautography. We used ddY strain mice of both sexes at various developmental and aging stages because of their small size. The livers, adrenal glands, lungs and testis were obtained from 3 sets of respective 10 groups of normal ddY strain mice, each consisting of 3 litter mates of both sexes, total 90, aged from embryonic day 19 to postnatal day 1, 3, 9 and 14 and month 1, 2, 6, 12 and 24. They were divided into 3 RI-groups, each consisting of 30 individuals, and administered with \(^3\)H-thymidine, \(^3\)H-uridine or \(^3\)H-leucine, respectively, then the livers, adrenal glands, lungs and testis were taken out after sacrifice under ether anesthesia and the tissues were processed for light and electron microscopic radioautography [38-40]. All the procedures used in this study concerning the animal experiments were in accordance with the guidelines of the animal research committee of Shinshu University School of Medicine, Matsumoto, Japan, where this experiment was carried out, as well as the principles of laboratory animal care in NIH publication No. 86-23 (revised 1985).
2.2. Light and Electron Microscopic Radioautography

All the animals were injected intraperitoneally with the RI-labeled precursors in saline, then the right medical lobes of the livers, the right adrenal glands, the right lower lobes of the lungs and the right testis were taken out, fixed in phosphate buffered glutaraldehyde and osmium tetroxide, embedded in epoxy resin Epok 812 (Oken, Tokyo, Japan), thick (2.0µm) or semithin (0.2µm) sections were cut on an MT-2B ultramicrotome. They were coated with Konica NR-M2 (LM) or NR-H2 (EM) radioautographic emulsions, exposed, developed and observed with either an Olympus Vanox light microscope or a JEOL JEM-4000EX (400kV) electron microscope [38-40].

3. Macromolecular Synthesis in Several Organs

3.1. The Liver

We first studied the liver tissues at various ages from embryo to postnatal 2 years [13-16, 41-50]. The results obtained from the tissues of 3 groups of animals injected separately with 3 kinds of RI-labeled precursors, $^3$H-thymidine, $^3$H-uridine and $^3$H-leucine were already reported as several original articles [41-48] or as a review in the previous series of this book [17]. Therefore, the results from the livers in aging mice are omitted in this article.

3.2. The Adrenal Gland

We studied the adrenal tissues of aging mice, both the adrenal cortex and the medulla, from embryo to postnatal 2 years. Some of the results were already published in several original articles [29, 30, 51-55].

3.2.1. Structure of Aging Mouse Adreno-Cortical Cells

We studied the adrenal tissues of mice at various ages from embryo to postnatal 2 years [51-55]. The adrenal tissues obtained from ddY strain mice at various ages from embryo day 19 to postnatal day 30 of both sexes, consisted of the adrenal cortex and the adrenal medulla [51]. The former consisted of 3 layers, zonaglomerulosa, zonafasciculata and zonareticularis, developing gradually with aging as observed by light microscopy (Fig. 1). At embryonic day 19 and postnatal day 1, the 3 layers of the adreno-cortical cells, zonaglomerulosa, zona fasciculate and zonareticularis were composed mainly of polygonal cells, while the specific orientation of the 3 layers was not yet well established. At postnatal day 3, orientation of 3 layers, especially the zonaglomerulosa became evident. At postnatal day 9 (Fig. 1) and 14, the specific structure of the 3 layers was completely formed and the arrangements of the cells in respective layers became typical especially at day 14 (Figs. 2-4) and month 1-24. Observing the ultrastructure of the adreno-cortical cells by electron microscopy, cell organelles including mitochondria were not so well developed at perinatal and early postnatal stages from embryonic day 19 to postnatal day 9. However, these cell organelles, mitochondria, endoplasmic reticulum, Golgi apparatus, appeared well developed similarly to the adult stages at postnatal day 14 (Figs. 2-4). The zonaglomerulosa (Fig. 2) is the thinnest layer found at the outer zone, covered by the capsule, consisted of closely packed groups of columnar or pyramidal cells forming arcades of cell columns. The cells contained many spherical mitochondria and well developed smooth surfaced endoplasmic reticulum but a compact Golgi apparatus in day 14 animals. The zonafasciculata (Fig.3) was the thickest layer, consisted of polygonal cells which were larger than the glomerulosa cells, arranged in long cords disposed radially to the medulla containing many lipid droplets. The mitochondria were less numerous and were more variable in size and shape than those of the glomerulosa cells, while the smooth surfaced endoplasmic reticulum were more developed and the Golgi apparatus was larger than the glomerulosa. In the zonareticularis (Fig. 4), the parallel arrangement of cell cords were anastomosed showing networks continued to the medullar cells. The mitochondria were less numerous and were more variable in size and shape than those of the glomerulosa cells like the fasciculata cells, as well as the smooth surfaced endoplasmic reticulum were developed and the Golgi apparatus was large like the fasciculata cells. However, the structure of the adrenal cortex tissues showed changes due to development and aging at respective developmental stages.
Fig. 1. LM radioautograms of a young mouse adrenal cortex at postnatal day 9, from left to right, zonaglomerulosa (33), fasciculata (29), and reticularis (30), labeled with $^3$H-thymidine, demonstrating DNA synthesis in the 3 zones of adrenal-cortical cells. Silver grains are localized over the nuclei in each zone (arrows). x1,000. From Nagata (2007), Proc. 6th Internat. Radioautography Symp., 1-25, Hebei Society Anat. Sci., 2007, Chengde, China.

Fig. 2. EMRAG of a juvenile mouse adrenal gland at postnatal day 14, labeled with $^3$H-thymidine, demonstrating DNA synthesis of adrenal-cortical cells in zona glomerulosa. Several silver grains are localized over several nuclei as well as over several mitochondria. x2,500. From Nagata (2007).

Fig. 3. EMRAG of a juvenile mouse adrenal gland at postnatal day 14, labeled with $^3$H-thymidine, demonstrating DNA synthesis of adrenal-cortical cells in zona fasciculata. Several silver grains are localized over the nucleus at right bottom as well as over several mitochondria. x5,000. From Nagata (2007).
3.2.2. DNA Synthesis in Aging Mouse Adreno-Cortical Cells

Observing both LM (Fig. 1) and EM (Figs. 2-4) radioautograms labeled with $^3$H-thymidine, demonstrating DNA synthesis, the silver grains were found over the nuclei of some adreno-cortical cells in S-phase of cell cycle mainly in perinatal stages at embryonic day 19, postnatal day 1 and day 3, while less at day 9 and day 14 (Figs. 2-4) to month 1-24 [29,49-52]. Those labeled cells were found in all the 3 layers, the zona glomerulosa (Figs. 1, 2), the zona fasciculata (Figs. 1, 3) and the zona reticularis (Figs. 1, 4), at respective aging stages. In labeled adreno-cortical cells in the 3 layers the silver grains were mainly localized over the euchromatin of the nuclei and only a few or several silver grains were found over the mitochondria of these cells (Fig. 4). To the contrary, most adreno-cortical cells were not labeled with any silver grains in their nuclei nor cytoplasm, showing no DNA synthesis after labeling with $^3$H-thymidine. Among many unlabeled adreno-cortical cells, however, most cells in the 3 layers were observed to be labeled with several silver grains over their mitochondria due to the incorporations of $^3$H-thymidine especially at the perinatal stages from embryonic day 19 to postnatal day 1, day 3, day 9 and 14 (Figs. 2-4). The ultrastructural localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices and some over the cristae or membranes (Fig. 3).

3.2.3. Number of Mitochondria of Aging Mouse Adreno-Cortical Cells

Preliminary quantitative analysis on the number of mitochondria in 10 adreno-cortical cells whose nuclei were labeled with silver grains and other 10 cells whose nuclei were not labeled in each aging group revealed that there was no significant difference between the number of mitochondria and the labeling indices ($P<0.01$). Thus, the number of mitochondria and the labeling indices were later calculated regardless whether their nuclei were labeled or not. The results obtained from the number of mitochondria in adreno-cortical cells in the 3 layers of respective animals in 10 aging groups at perinatal stages, prenatal embryo day 19, postnatal day 1, 3, 9 and 14, showed an gradual increase from the prenatal day 19 (glomerulosa 12.5, fasciculata 14.9, reticularis 15.2/cell) to postnatal day 14 and month 1 and 2 (glomerulosa 62.2, fasciculata 64.0, reticularis 68.2/cell). The increase from embryo day 19 to postnatal month 2 was stochastically significant ($P <0.01$). Then, they did not change significantly to month 12 and 24.

3.2.4. Mitochondrial DNA Synthesis of Aging Mouse Adreno-Cortical Cells

The results of visual grain counts on the number of mitochondria labeled with silver grains obtained from 10 adreno-cortical cells in the 3 layers of each animal labeled with $^3$H-thymidine demonstrating DNA synthesis in 10 aging groups at perinatal stages, prenatal embryo day 19, postnatal day 1, 3, 9 and 14, and month 1, 2, 6, 12 and 24 were reported previously [29,51-55]. The results demonstrated that the numbers of labeled mitochondria with $^3$H-thymidine showing DNA synthesis gradually increased from prenatal embryo day 19 (glomerulosa 0.3, fasciculata 0.5, reticularis 0.4/cell) to postnatal day 14, month 1 and 2 (glomerulosa 5.3, fasciculata 5.0, reticularis 6.2/cell), reaching the maximum, then decreased to month 6, 12 and 24. The increase and decrease were stochastically significant ($P <0.01$).

3.2.5. The Labeling Index of DNA Synthesis in Mouse Adreno-Cortical Mitochondria

On the other hand, the labeling indices in respective aging stages were calculated from the number of labeled mitochondria, dividing by the number of total mitochondria per cell which were mentioned previously.
The results showed that the labeling indices gradually increased from prenatal day 19 (glomerulosa 2.4, fasciculata 2.7, reticularis 2.6%) to postnatal day 14, month 1 and 2 (glomerulosa 8.5, fasciculata 7.8, reticularis 8.8%), reaching the maximum and decreased to month 24. The increase and decrease were stochastically significant (P <0.01).

3.2.6. Mitochondrial RNA Synthesis of Aging Mouse Adreno-Cortical Cells

We also studied the adrenal tissues of mice at various ages from embryo to postnatal 1 year labeled with $^3$H-uridine [30, 55]. The adrenal tissues obtained from ddY strain mice in 10 groups, each consisting of 3 individuals at various ages from embryo day 19 to postnatal day 1, 3, 8, 14, 30, month 2, 6 and 12, were studied by light and electron microscopic radioautography.

The adrenal cortex of each animal labeled with $^3$H-uridine consisted of 3 layers, zona glomerulosa, zona fasciculata and zona reticularis, developing gradually with aging as was observed by $^3$H-uridine radioautography. At embryonic day 19 and postnatal day 1, the adreno-cortical cells were composed mainly of polygonal cells, but the specific orientation of the 3 layers, zona glomerulosa, zona fasciculata and zona reticularis, was not yet well established. At postnatal day 3 on the orientation of 3 layers became evident. At postnatal day 9 and 14, the specific structure of 3 layers was completely formed and the arrangements of the cells in respective layer became typical especially at day 14 and month 1. Observing the ultrastructure of the adreno-cortical cells by electron microscopy, cell organelles including mitochondria were not so well developed at perinatal and early postnatal stages from embryonic day 19 to postnatal day 1, day 3 and day 9. Then, these cell organelles, mitochondria, endoplasmic reticulum, GOLGI apparatus, appeared well developed similarly to the adult stages at postnatal day 14 (Fig. 5), and the 3 layers of the adrenal cortex, as well as 2 cell types of the adrenal medulla in adult stages, from postnatal month 1 to 6, developed well due to aging. The number of mitochondria per cell and the number of labeled mitochondrial per cell gradually increased due to aging.

Fig. 5. EMRAG of a juvenile mouse adrenal gland at postnatal day 14, labeled with $^3$H-uridine, demonstrating RNA synthesis of adreno-cortical cells in zona glomerulosa. Several silver grains are localized over several nuclei as well as over several mitochondria. x2,500. From Nagata (2007).

For quantitative analysis of electron micrographs, 10 EM radioautograms showing cross sections of adreno-cortical cells in the 3 zones, glomerulosa, fasciculate and reticularis, based on the electron microscopic photographs taken after observation on at least 100 adreno-cortical cells from respective animals, were analyzed for calculating the total number of mitochondria in each adreno-cortical cell and the number of labeled mitochondria covered with silver grains by visual grain counting [30]. The results obtained from the number of mitochondria in adreno-cortical cells in the 3 layers of respective animals in 3 aging groups at postnatal day 14, month 6 and 12, showed an gradual increase from postnatal day 14 (25.1~23.0/cell), to adult stages at postnatal month 6 (42.4~37.8/cell) and to month 12 (54.7~50.2/cell). The increase from postnatal day 14 to postnatal month 12 was stochastically significant (P <0.01).

On the other hand, the results of visual grain counting on the number of mitochondria labeled with silver grains obtained from 10 adreno-cortical cells in the 3 layers of each animal labeled with $^3$H-uridine demonstrating RNA synthesis in 3 aging groups at postnatal day 14, month 6 and 12 showed that the numbers of labeled mitochondria with $^3$H-uridine per cell gradually increased from postnatal day 14 (1.3~1.7/cell) to month 6 (3.1~4.5/cell) and month 12 (7.1~9.5/cell). The increase was also stochastically significant (P <0.01).
3.2.7. Labeling Index of RNA Synthesis in Mitochondria of Mouse Adreno-Cortical Cells

From the above results, the labeling indices in respective aging stages were calculated from the number of labeled mitochondria dividing by the number of total mitochondria per cell which showed that the labeling indices of 3 layers gradually increased from juvenile stage at postnatal day 14 (5.1–7.3%) to month 6 (7.3–11.1%) and 12 (9.5–14.1%). The increase was also stochastically significant (P <0.01).

3.2.8. Mitochondrial Protein Synthesis of Aging Mouse Adreno-Cortical Cells

We studied protein synthesis in mitochondria of aging mouse adreno-cortical cells after administration of RI-labeled amino-acids such as $^3$H-leucine which is one of the protein precursors. The silver grains in radioautograms due to $^3$H-leucine were found over the adreno-cortical cells in 3 layers. The number of labeled cells and the labeling index in all the layers increased and decreased due to aging. The quantitative analysis on these studies are now in progress.

3.2.9. DNA, RNA and Protein Synthesis of Aging Mouse Adreno-Medullary Cells

The medullary cells of mouse consist of both adrenalin cells and noradrenalin cells (Figs. 6, 7). When they were labeled with macromolecular precursors such as $^3$H-thymidine (Fig. 6), $^3$H-uridine (Fig. 7) or $^3$H-leucine and radioautographed, the silver grains were found over both the adrenalin cells and noradrenalin cells (Fig. 6, 7). The labeling indices in both the cell types labeled with $^3$H-thymidine increased and decreased due to aging [54]. The quantitative analysis on these studies dealing with the incorporations of $^3$H-uridine and $^3$H-leucine are now in progress [55].

Fig. 6. EMRAG of a juvenile mouse adrenal gland at postnatal day 14, labeled with $^3$H-thymidine, demonstrating DNA synthesis of adreno-medullary cells. Several silver grains are localized over the nuclei as well as over several mitochondria in both adrenalin and noradrenalin cells. x5,000. From Nagata (2007).

Fig. 7. EMRAG of a juvenile mouse adrenal gland at postnatal day 14, labeled with $^3$H-uridine, demonstrating RNA synthesis of adreno-medullary cells. Several silver grains are localized over several nuclei as well as over several mitochondria of both the cell types, adrenalin cells and noradrenalin cells. x5,000. From Nagata (2007).
3.3. The Lung

We studied the pulmonary tissues as well as the trachea at various ages from embryo to postnatal 2 years [20-22, 55]. The pulmonary tissues obtained from ddY strain mice at various ages from embryo day 19 to adult postnatal day 30 and to year 2 consisted of several types of cells, i.e., the type I epithelial cells, the small alveolar epithelial cells, type II epithelial cells, large alveolar epithelial cells, interstitial cells and endothelial cells, which incorporated macromolecular precursors respectively (Figs. 8-11).

3.3.1. Structure of Aging Mouse Lung

The pulmonary tissues obtained from ddY strain mice at embryonic day 19 to early postnatal stages consisted of undifferentiated cells. However, they differentiated into several types of cells due to aging, the type I epithelial cells (Fig. 8) or the small alveolar epithelial cells, the type II epithelial cells (Fig. 9) or the large alveolar epithelial cells, the interstitial cells (Fig. 10), the endothelial cells and the alveolar phagocyte or dust cell as we had formerly reported [20-22]. At embryonic day 16 and 18, the fetal lung tissues appeared as glandular organizations consisting of many alveoli bordering undifferentiated cuboidal cells and no squamous epithelial cells were seen. Mitotic figures were frequently observed in cuboidal epithelial cells. After birth, the structure of the alveoli was characterized by further development of the alveolar-capillary networks from postnatal day 1 to 3 and 9. During the development, the cellular composition of the alveolar epithelium resembled that of the adult lung, with a mixed population of the type I and type II epithelial cells. Up to 1 and 2 weeks after birth, the lung tissues showed complete alveolar structure and single capillary system almost the same as the adult after 1 month to 2 to 6 months, and further to senescent stage over 12 months to 24 months.

Fig. 8. EMRAG of the type I epithelial cell of an adult mouse lung at postnatal month 1, labeled with 3H-thymidine, demonstrating DNA synthesis. Silver grains are localized over 2 nuclei and a few mitochondria. x5,000. From Nagata (2007).
3.3.2. Mitochondrial DNA Synthesis of Aging Mouse Pulmonary Cells

On electron microscopic radioautograms of the pulmonary tissues labeled with \( ^3 \)H-thymidine, silver grains were observed over the nuclei of some pulmonary cells corresponding to the DNA synthesis in S-phase as observed by light microscopic radioautography [20,22]. On the other hand, some mitochondria in both S-phase cells and interphase cells which did not show any silver grains over their nuclei were labeled with silver grains showing intramitochondrial DNA synthesis. The intramitochondrial DNA synthesis was observed in all the cell types, the type I epithelial cell (Fig. 8), the type II epithelial cell (Fig. 9), the interstitial cell (Fig. 10) and the endothelial cell. Because enough numbers of electron photographs (more than 5) were not obtained from all the cell types in respective aging groups, only some cell types and some aging groups when enough numbers of electron photographs were available were used for quantitative analysis. The numbers of mitochondria per cell profile area, the numbers of labeled mitochondria per cell and the labeling indices of the type I epithelial cells in only a few aging groups were observed and counted [20, 22]. The labeling indices in respective aging stages were calculated from the number of labeled mitochondria and the number of total mitochondria per cellular profile area which were calculated, respectively. These results demonstrated that the labeling indices in
these cell types decreased from prenatal stages at embryo day 16 to day 18 (20-25%), and further decreased to postnatal days up to senescent stages due to aging [20, 22].

3.3.3. Mitochondrial RNA Synthesis of Aging Mouse Pulmonary Cells

On electron microscopic radioautograms of pulmonary tissues labeled with $^3$H-uridine, silver grains were observed over the nuclei of some pulmonary cells (Fig. 11) corresponding to the RNA synthesis in most cells in respective aging groups as observed by light and electron microscopic radioautography. The silver grains were observed to localize not only over euchromatin and nucleoli in the nuclei but also over many cell organelles such as endoplasmic reticulum, ribosomes, and mitochondria as well as cytoplasmic matrices of all the cell types [21]. The intramitochondrial RNA synthesis was observed in all the cell types, the type I epithelial cell, the type II epithelial cell (Fig. 11), the interstitial cell and the endothelial cell. Because enough numbers of electron radioautograms (more than 5) were not obtained from all the cell types in respective aging groups, only some cell types and some aging groups when enough numbers of electron photographs were available were used for quantitative analysis similarly to DNA synthesis. The numbers of mitochondria per cell profile area, the numbers of labeled mitochondria per cell and the labeling indices of the type I epithelial cells in only a few aging groups were counted. Likewise, the similar results from the type II epithelial cells, the interstitial cells, and the endothelial cell were counted. The labeling indices in respective aging stages were calculated from the number of labeled mitochondria and the number of total mitochondria per cellular profile area, respectively. These results demonstrated that the numbers of labeled mitochondria in these cell types increased from perinatal stages to the adult stage, reaching the maxima (20-25%) at postnatal month 1, and decreased to the senescent stage due to aging.

![RNA type II epithelial cell](image.png)

Fig. 11. EMRAG of the type II epithelial cell of an adult mouse lung at postnatal month 1, labeled with $^3$H-uridine, demonstrating RNA synthesis. Several silver grains are localized over the 2 nuclei at center and right as well as several mitochondria. x5,000. From Nagata (2007).

3.4. The Testis

3.4.1. DNA Synthesis in Aging Mouse Testis

We studied the macromolecular synthesis in the testis of aging male ddY mice at various ages [24-26,55]. When testicular tissues were labeled with $^3$H-thymidine and observed by LM and EM radioautography, many spermatogonia and myoid cells as well as Leydig cells were labeled with $^3$H-thymidine at various ages from embryonic day 19 to postnatal day 1, 3, 9, 14 (Fig. 12), month 1, 2, 6, 12 and 24. Silver grains were localized over the nuclei and several mitochondria of the spermatogonia showing DNA synthesis. Among of the aging groups, we counted the numbers of mitochondria per cell profile area, the numbers of labeled mitochondria per cell of the spermatogonia from 4 aging groups, prenatal embryonic day 19, postnatal day 3, month 1 and 6, and the labeling indices were calculated. The results
showed that the LI of the spermatogonia increased from embryonic day 19 (17%) to postnatal month 1, reaching the maximum (30%), then decreased to month 6 (25%).

3.4.2. RNA Synthesis in Aging Mouse Testis

We also studied RNA synthesis in the testis of aging ddY mice at various ages [24, 25, 55]. When testicular tissues were labeled with \(^{3}\)H-uridine and observed by LM and EM radioautography, many spermatogonia and myoid cells as well as Sertoli cells and Leydig cells were labeled with \(^{3}\)H-uridine at various ages from embryonic day 19 to postnatal day 1, 3, 7, 14, month 1, 3, 6, 12 and 24. Silver grains were localized over the nuclei and several mitochondria of the spermatogonia, myoid cells, Sertoli cells and Leydig cells showing RNA synthesis. Among of the aging groups, from prenatal day 19, postnatal day 3, month 3, 6, 12 and 24, we counted the numbers of silver grains in nuclei and cytoplasm in spermatogonia, myoid cells and Sertoli cells [24]. The incorporation increased from month 3 and reached the maximum at month 6, then decreased to month 12. However, the number of labeled mitochondria per cell profile area and the labeling index were not yet analyzed.

3.4.3. Protein Synthesis in Aging Mouse Testis

We also studied protein synthesis in the testis of aging ddY mice by \(^{3}\)H-leucine incorporations at various ages [24, 25, 55]. When testicular tissues were labeled with \(^{3}\)H-leucine and observed by LM and EM radioautography, many spermatogonia and myoid cells as well as Sertoli cells were labeled with \(^{3}\)H-leucine at various ages from embryonic day 19 to postnatal day 1, 3, 9, 14, month 1, 3, 6, 12 and 24. Silver grains were localized over the nuclei and several mitochondria of the spermatogonia, myoid cells and Sertoli cells showing protein synthesis. The incorporation increased from month 3 and reached the maximum at month 6 to 12. However, the number of labeled mitochondria per cell profile area and the labeling index were not yet analyzed.

4. Concluding Remarks

From the results obtained at present, nucleic acids, both DNA and RNA, and protein synthesis showing incorporations of \(^{3}\)H-thymidine, \(^{3}\)H-uridine and \(^{3}\)H-leucine were demonstrated in the nuclei and mitochondria of hepatocytes of the liver, adreno-cortical cells and adreno-medullary cells of the adrenal glands, 4 types of pulmonary cells of the lung and spermatogonia, myoid cells and Leydig cells of the testis of ddY mice at various ages from fetal to postnatal newborn, juvenile, young, adult and senescent individuals.

The numbers of mitochondria per cell, the numbers of labeled mitochondria per cell and the labeling indices of hepatocytes, adreno-cortical and medullary cells, pulmonary cells and spermatogonia at various ages changed due to aging, increases and decreases, independently from the nuclear macromolecular syntheses. These results indicate that the mitochondria in respective cell types of these organs synthesize DNA, RNA and proteins by themselves, increase their numbers per cell with the aging of the individual animals. These results form a part of the systematic studies on special cytochemistry [37] and radioautographology [38] as was formerly proposed by the author [37, 38, 55].
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