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Macromolecular Synthesis in the Endocrine, Nervous and Sensory Systems

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1. Introduction

This chapter deals with the last forth part of the application of microscopic radioautography to the organ systems, including the endocrine system, the nervous system and the sensory organs. The endocrine system includes the hypophysis, the pineal body, the thyroid gland, the parathyroid gland, the thymus, the adrenal gland, the islet of Langerhans and the reproductive glands, i. e. the testis and the ovary. The nervous system includes the central nervous system, i.e. the brains and spinal cord and the peripheral nerves, i.e. the ganglion and nerves, while the sensory system includes, the skin, the visual, the stato-acoustic, the olfactory and the gustatory organs. We have studied some of these organs, not all of them yet.

2. Macromolecular synthesis in the endocrine system

Among the endocrine organs, we studied macromolecular synthesis in the adrenal gland and the steroid secreting cells of both sexes, the Leydig cells of the testis and the ovarian follicular cells in mice. On the other hand, incorporation of mercury chloride into the human thyroid tissues was also studied (Nagata 2002).

2.1 The DNA synthesis in the endocrine system

Among the endocrine organs, we studied DNA synthesis in the adrenal glands and steroid secreting cells of both sexes, the Leydig cells of the testis and the ovarian follicular cells in mice.

2.1.1 The DNA synthesis in the adrenal gland

We studied the adrenal tissues of aging mice, both the adrenal cortex and the medulla, from embryo to postnatal 2 years in senescence. Some of the results were already published in several original articles (Ito 1996, Ito and Nagata 1996, Liang 1998, Liang et al. 1999, Nagata 1994, 1999c, 2000a,b, 2008a,b, 2009c,d,e,f,g,h,i,j, 2010a, Nagata et al. 2000b). The results shall be summarized in this review.

2.1.1.1 The DNA synthesis in the adrenal cortex

We studied the adrenal tissues of mice at various ages from embryo to postnatal 2 years (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b, 2009c,d,e,f,g,h,i,j). 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. The former consisted of 3 layers, zona glomerulosa (Fig. 20A), zona fasciculata (Fig. 20B) and zona reticularis (Fig. 20C), developing gradually with aging from perinatal stage at embryonic day 19 to postnatal stages as day 1, 3, 9, 14, month 1, 2, 6, 12, 24 as observed by light microscopy.

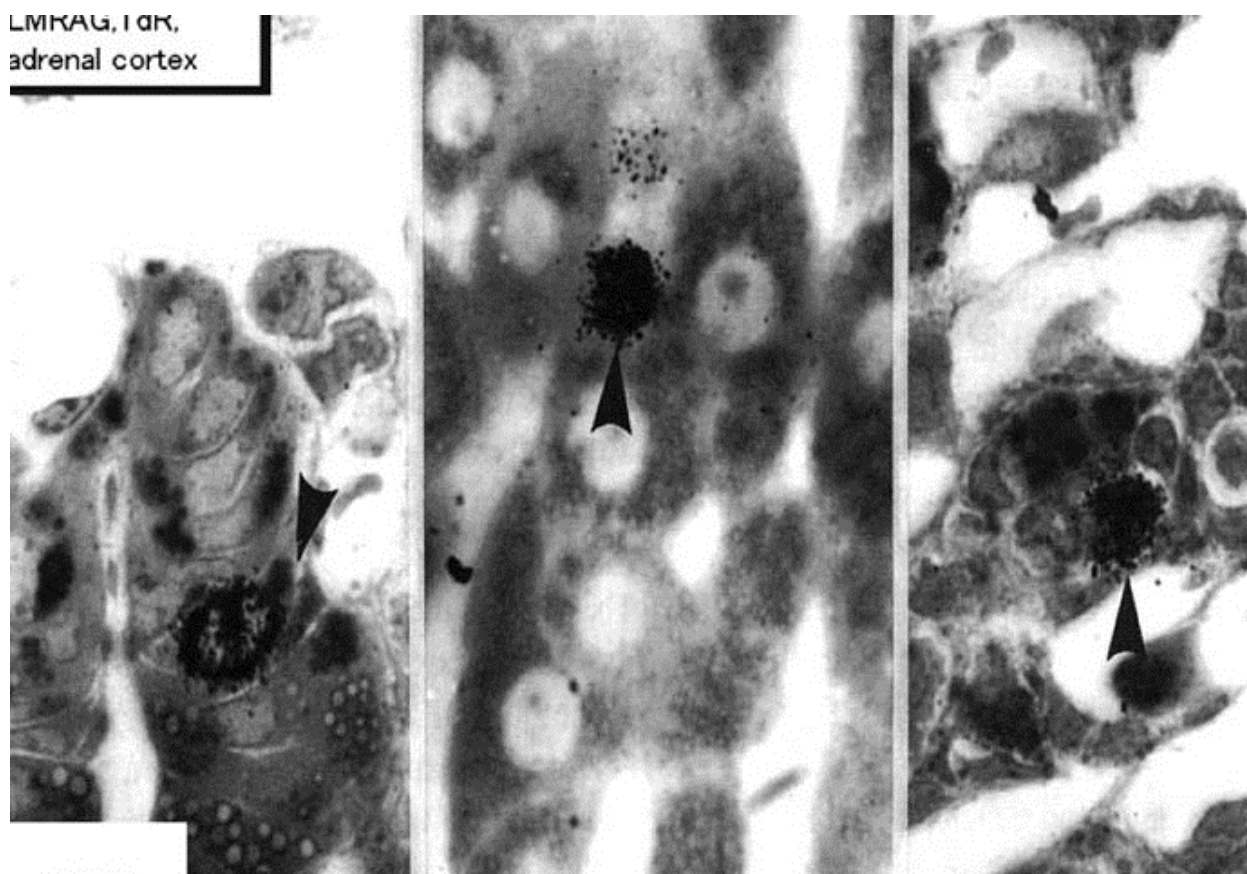


Fig. 20. LM RAG of a young mouse adrenal cortex, labeled with ^3H -thymine, showing DNA synthesis (arrow) in 3 layers, zona glomerulosa (left), zona fasciculata (middle), zona reticularis (right). From Nagata, T.: *Annals Microsc.* Vol. 10, p. 61, 2010, Microsc. Soc. Singapore.

At embryonic day 19 and postnatal day 1, the 3 layers of the adreno-cortical cells, zona glomerulosa (Fig. 21D), zona fasciculata (Fig. 21E) and zona reticularis were composed mainly of polygonal cells, while the specific orientation of the 3 layers was not yet well

established as observed by EMRAG. At postnatal day 3, orientation of 3 layers, especially the zona glomerulosa became evident. At postnatal day 9 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 (Fig. 21D) and month 1 (Fig. 21E) to 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. The zona glomerulosa 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 zona fasciculata was the thickest layer, consisted of polygonal cells that 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 zona reticularis, 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.

Observing both LM and EM RAG of the adrenal cortex labeled with ^3H -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 to month 1-24 (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b,c,d, 2009c,d,e). Those labeled cells were found in all the 3 layers (Fig. 20), the zona glomerulosa (Fig. 20 left), the zona fasciculata (Fig. 20 middle) and the zona reticularis (Fig. 20 right), 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 as observed by EM RAG (Fig. 21D,E).

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 ^3H -thymidine. The labeling indices in respective 3 zones in the cortex as well as the medulla showed the maxima at perinatal stages and decreased due to aging (Fig. 22A,B). 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 ^3H -thymidine especially at the perinatal stages from embryonic day 19 to postnatal day 1, day 3, day 9 and 14 (Fig. 21D). The ultrastructural localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices and some over the cristae or membranes.

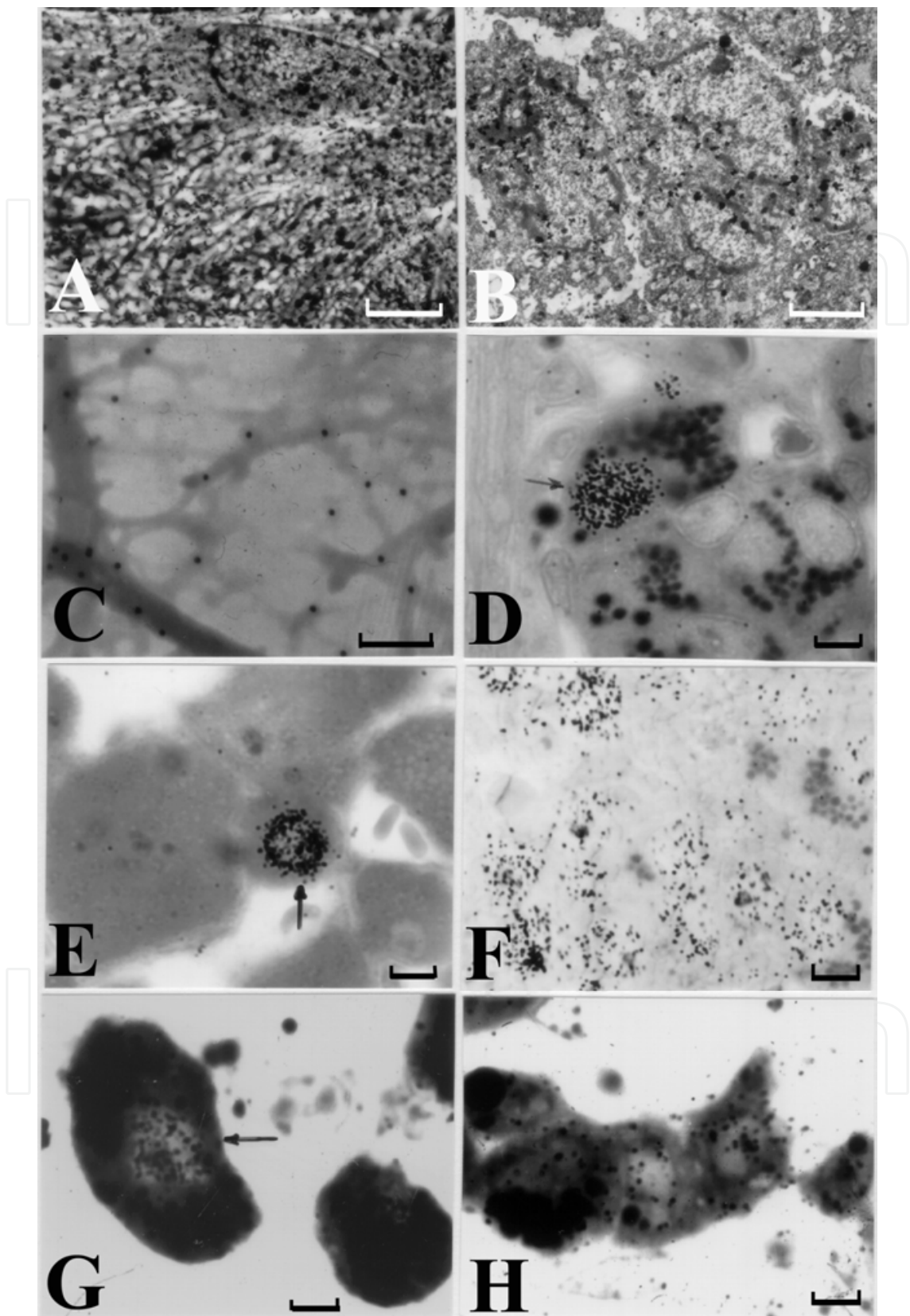


Fig. 21. LM and EM RAG of the endocrine organs. From Nagata, T.: Special Cytochemistry in Cell Biology, In, Internat. Rev. Cytol. Vol. 211, No. 1, p. 115, 2001, Academic Press, San Diego, USA, London, UK.

Fig. 21A. EM RAG of human thyroid cancer cells, labeled with $^{205}\text{HgCl}_2$ in vitro, quick frozen, freeze-dried, embedded in Epoxy resin, dry-sectioned, and radioautographed by dry-mounting procedure for demonstrating soluble compounds, many silver grains showing soluble $^{205}\text{HgCl}_2$ incorporations. $\times 15,000$.

Fig. 21B. EM RAG of human thyroid cancer cells, labeled with $^{205}\text{HgCl}_2$ in vitro, chemically fixed doubly in buffered glutaraldehyde and osmium tetroxide, dehydrated, embedded in Epoxy resin, wet-sectioned, and radioautographed by wet-mounting procedure for demonstrating insoluble compounds, less silver grains showing insoluble $^{205}\text{HgCl}_2$ incorporations. $\times 15,000$.

Fig. 21C. EM photo of a human thyroid cancer cell, fixed in paraformaldehyde and glutaraldehyde mixture, embedded in Lowicryl K4M, sectioned and immuno-stained with anti-keratin antibody by the protein-A gold technique, demonstrating keratin filaments. $\times 15,000$.

Fig. 21D. LM RAG of the zona glomerulosa of the adrenal cortex of a postnatal day 14 mouse labeled with ^3H -thymidine, showing DNA synthesis. $\times 900$.

Fig. 21E. LM RAG of the zona fasciculata of the adrenal cortex of a postnatal month 6 mouse labeled with ^3H -thymidine, showing DNA synthesis. $\times 900$.

Fig. 21F. LM RAG of the zona glomerulosa of the adrenal cortex of a prenatal day 19 mouse labeled with ^3H -uridine, showing RNA synthesis. $\times 1,000$.

Fig. 21G. LM RAG of the interstitial tissues of a postnatal month 12 male mouse labeled with ^3H -thymidine, showing DNA synthesis in Leydig cells. $\times 1,000$.

Fig. 21H. LM RAG of the interstitial tissues of a postnatal day 3 male mouse labeled with ^3H -uridine, showing RNA synthesis in Leydig cells. $\times 1,000$.

2.1.1.1.1 The number of mitochondria of 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 (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b,c,d, 2009c,d,e). 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, 2 (glomerulosa 62.2, fasciculata 64.0, reticularis 68.2/cell) to month 6 and 12. The increase from embryo day 19 to postnatal month 2 was stochastically significant ($P < 0.01$). Then, they did not change significantly from month 12 to 24 (Fig. 22A).

2.1.1.1.2 The DNA synthesis in the mitochondria of 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 ^3H -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 (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b, 2009c,d,e). The results demonstrated that the numbers of labeled mitochondria with ^3H -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 (Fig. 22A). The increase and decrease were stochastically significant ($P < 0.01$).

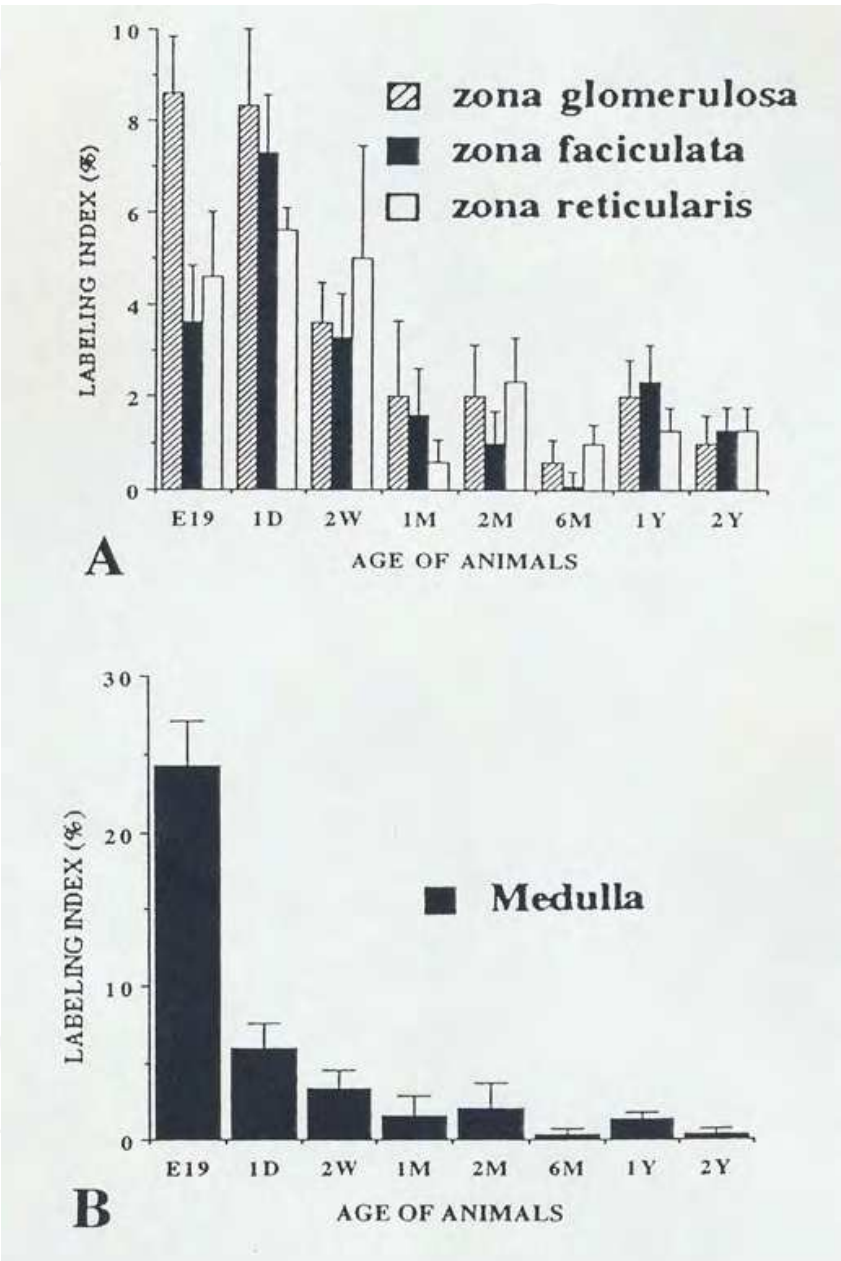


Fig. 22. Histogram showing aging changes of average labeling indices in respective cell types of the adrenal glands of aging mice labeled with ^3H -thymidine showing DNA synthesis. Mean \pm Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 171, 2002, Urban & Fischer, Jena, Germany

Fig. 22A. The adrenal cortex.

Fig. 22B. The adrenal medulla.

2.1.1.1.3 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 (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b, 2009c,d,e,j, 2010e). 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 6 (glomerulosa 4.1, fasciculata 4.2, reticularis 3.8%), 12 and 24 (Fig. 23C). The increase and decrease were stochastically significant ($P < 0.01$).

2.1.1.2 The DNA synthesis in mouse adreno-medullary cells

We studied the adrenal tissues of mice at various ages from embryo to postnatal 2 years. 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 (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b, 2009c,d,e,g, 2010d,e,f,g). The former consisted of 3 layers, zona glomerulosa, zona fasciculata and zona reticularis, developing gradually with aging as observed by light microscopy (Fig. 20), while the latter consisted of 2 cell types in one layer when observed by electron microscopy (Nagata 2009c,d,e, 2010d,e,f,g). At embryonic day 19 and postnatal day 1, the 3 layers of the adreno-cortical cells, zona glomerulosa, zona fasciculate and zona reticularis were composed mainly of polygonal cells, while the specific orientation of the 3 layers was not yet well established. However, the orientation of 3 layers became evident at day 3 and completely formed at day 14 (Fig. 20) and to month 1-24 (Fig. 21D,E,F). On the other hand, the medulla consisted of only one layer containing 2 types of cells, adrenalin cell and noradrenalin cell. The former contains adrenalin granules with low electron density, while the latter contains noradrenalin granules with high electron density.

The adrenal medulla is the deepest layer in the adrenal glands, surrounded by the 3 layers of the adrenal cortex as observed by light and electron microscopy (Ito 1996, Ito and Nagata 1996, Nagata 2008a,b,c,d, 2009c,d,e, 2010d,e,f,g), containing either adrenalin granules or noradrenalin granules. Quantitative analysis revealed that the numbers of mitochondria in both adrenalin and noradrenalin cells at various ages increased from fetal day 19 to postnatal month 1 due to aging of animals, respectively, but did not decrease to month 24, while the number of labeled mitochondria and the labeling indices of intramitochondrial DNA synthesis changed due to aging. When they were labeled with ^3H -thymidine silver grains appeared over some nuclei of both cell types at perinatal stages, but they appeared almost all the cell bodies containing mitochondria. Quantitative analysis revealed that the numbers of mitochondria in both adrenalin and noradrenalin cells at various ages increased from fetal day 19 to postnatal month 1 due to aging of animals, respectively, while the number of labeled mitochondria and the labeling indices of intramitochondrial DNA synthesis incorporating ^3H -thymidine increased from fetal day 19 to postnatal day 14 (2 weeks), reaching the maxima, and decreased to month 24. It was shown that the activity of intramitochondrial DNA synthesis in the adrenal medullary cells in aging mice increased and decreased due to aging of animals.

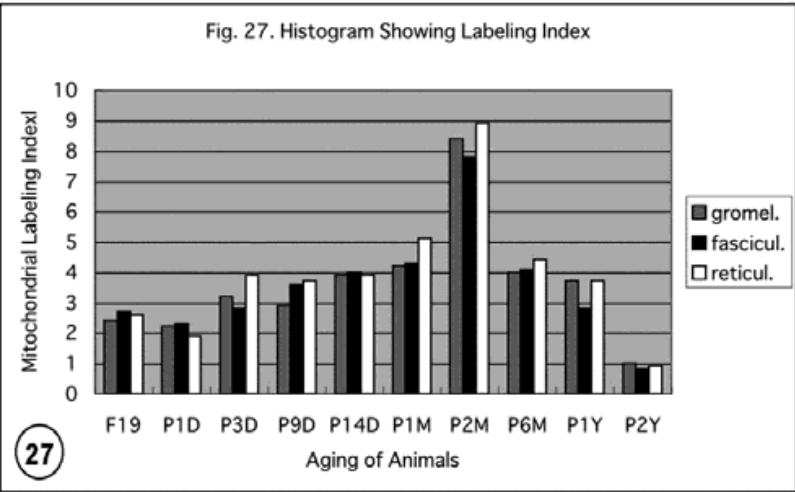
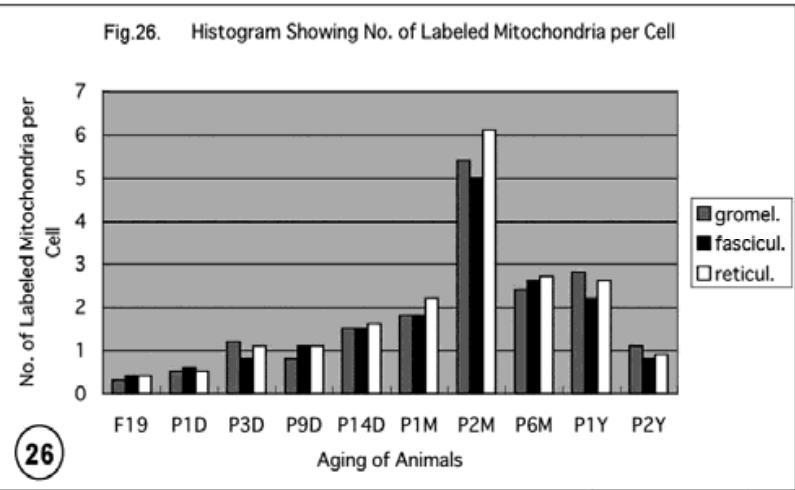
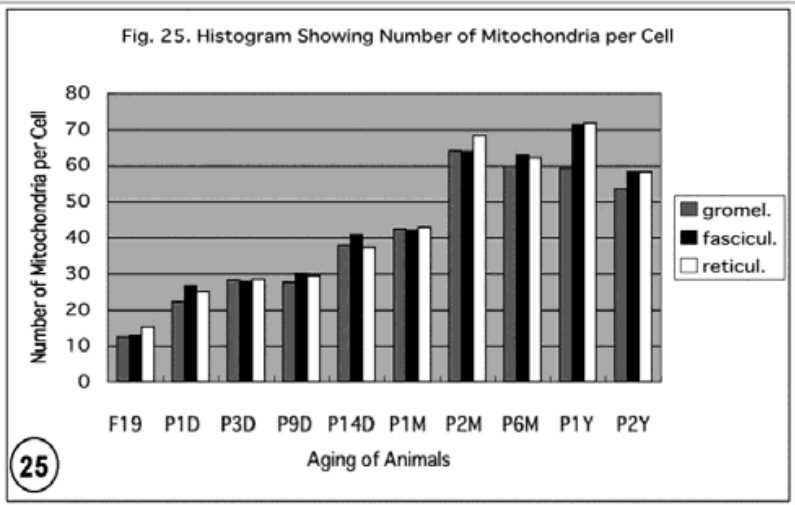


Fig. 23. Histogram showing aging changes of the respective cell types of the adrenal glands of aging mice labeled with ^3H -thymidine showing DNA synthesis. Mean \pm Standard Deviation. From Nagata, T.: Current Radiopharmaceutics, Vol. 2, p. 173, 2002.
Fig. 23A(25). The number of mitochondria per cell.
Fig. 23B(26). The number of labeled mitochondria per cell.
Fig. 23C(27). The mitochondrial labeling index.

2.1.2 The DNA synthesis in the islets of Langerhans

When we studied macromolecular synthesis in the exocrine pancreatic cells of aging mice by LM and EMRAG we also studied the islet cells of Langerhans together with the exocrine cells, using RI labeled precursors such as ^3H -thymidine for DNA (Nagata and Usuda 1985, 1986, Nagata et al. 1986a,b), ^3H -uridine for RNA (Nagata and Usuda 1985, 1993b, Nagata et al. 1986a,b), ^3H -leucine for proteins (Nagata 2000, Nagata and Usuda 1993a, 1995), ^3H -glucosamine for glucides (Nagata et al. 1992), ^3H -glycerol for lipids (Nagata et al. 1988b, 1990). The results showed that the islets cells, A, B and C cells, incorporated those precursors to synthesize DNA, RNA, proteins and glucides. The labeling index of DNA synthesis and the densities of silver grains showing RNA, proteins and glucides syntheses were high at prenatal and earlier postnatal stages from day 1 to day 14, then decreased from 1 month to 1 years due to aging. However, the labeling indices by ^3H -thymidine and the grain counts by ^3H -uridine and ^3H -leucine in the endocrine cells were less than those in the exocrine cells at the same ages.

2.1.3 The DNA synthesis in the Leydig cells of the testis

The cells of Leydig can be found in the interstitial tissues between the seminiferous tubules of the testis of mammals (Gao 1993, Gao et al. 1994, 1995a,b, Nagata et al. 2000b). They are identified as spherical, oval, or irregular in shape and their cytoplasm contains lipid droplets. We studied the macromolecular synthesis of the cells in the testis of several groups of litter ddY mice at various ages from fetal day 19 to postnatal aging stages up to 2 years senescence by LM and EMRAG using ^3H -thymidine, ^3H -uridine and ^3H -leucine incorporations.

The Leydig cells from embryonic stage to senescent stages were labeled with ^3H -thymidine as observed by LMRAG (Fig. 21G). The changes of the numbers of labeled Leydig cells with the ^3H -thymidine incorporation into the nuclei showing the DNA synthesis were found in these cells at different aging stages. Only a few cells were labeled after ^3H -thymidine at embryonic day 19. At early postnatal stages, there was a slight increase of the number of labeled cells. The number of labeled cells from perinatal stage to postnatal 14 days and 1, 2, 6 months were similar to the values found at prenatal and early postnatal stages. The notable increases in the number of labeled cells of Leydig were found from 9 months to 2 years in senescence. The labeling indices with ^3H -thymidine in perinatal stages to postnatal 6 months were low (5-10%) but increased at 9 months and maintained high level (50-60%) to 2 years (Gao 1993, Gao et al. 1994, 1995a, Nagata et al. 2000b). The labeling indices at senescent stages still maintained a relatively high level and they were obviously higher than those of young animals. By electron microscopy, typical Leydig cells contained abundant cell organelles such as smooth surfaced endoplasmic reticulum, Golgi apparatus and mitochondria with tubular cristae. The silver grains were mainly localized over the euchromatin of labeled nucleus. Some of the grains were also localized over some of the mitochondria in both the nuclei labeled and unlabeled cells.

2.1.4 The DNA synthesis in the ovarian follicles

The ovarian follicles in the ovaries of mature mice are one of the steroid secreting organs in female animals. We studied the DNA and RNA synthesis of the follicular cells in the developing ddY mice ovaries in several aging groups at postnatal day 1, 3, 7, 14, 30 and 60 by LM and EMRAG using ^3H -thymidine and ^3H -uridine (Li 1994, Li and Nagata 1995). From

the results it was shown that both DNA and RNA synthesis in the ovarian follicular cells were observed (Fig. 16G). Quantitative analysis, as expressed with labeling indices and grain counts, revealed that both increased significantly from postnatal day 1 to 3, then decreased from day 7 to 60 (Fig. 19A). Comparing the results to other female genital cells, a paralleled alteration of both DNA and RNA synthesis was revealed between the ovarian follicular cells and other uterine or oviductal cells (Fig. 19B,C). On the other hand, the glycoconjugate synthesis as shown by the uptake of $^{35}\text{SO}_4$ in mouse ovary during the estrus cycle was also demonstrated (Li et al. 1992).

2.2 The RNA synthesis in the endocrine system

We studied the RNA synthesis of the adrenal glands and the cells of Leydig in the testis of aging mice among the endocrine organs after the administration of ^3H -uridine at various ages.

2.2.1 The RNA synthesis in the adrenal glands

The RNA synthesis in the adrenal glands in aging mice was studied in both the adrenal cortex and the adrenal medulla after administration of ^3H -uridine in many groups of mice at various ages from perinatal stages to senescence at year 2.

2.2.1.1 The RNA synthesis in aging mouse adreno-cortical cells

Observing both LM and EM RAG labeled with ^3H -uridine, demonstrating RNA synthesis, the silver grains were found over the nuclei and cytoplasm of almost all the adreno-cortical cells from perinatal stages to postnatal month 1-24 (Liang 1998, Liang et al. 1999, Nagata et al. 2000b, Nagata 2010a). Those labeled cells were found in all the 3 layers, the zona glomerulosa (Fig. 21F), the zona fasciculata and the zona reticularis, 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 several silver grains were found over the endoplasmic reticulum, ribosomes and mitochondria of these cells. The ultrastructural localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices and some over the cristae or membranes.

2.2.1.2 The RNA synthesis in the mitochondria of 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 ^3H -uridine demonstrating RNA 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 (Liang 1998, Liang et al. 1999, Nagata et al. 2000b, Nagata 2010a). The results demonstrated that the numbers of labeled mitochondria with ^3H -uridine showing RNA 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 (Fig. 23B). The increase and decrease were stochastically significant ($P < 0.01$).

2.2.1.3 The labeling index of RNA 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 (Fig. 14B), dividing by the number of total mitochondria per cell (Fig. 24A) which were mentioned previously (Liang 1998, Liang et al. 1999, Nagata

et al. 2000b, Nagata 2010a). The results showed that the labeling indices gradually increased from prenatal day 19 (glomerulosa 10.4, fasciculata 12.1, reticularis 13.1%) to postnatal day 1 (glomerulosa 12.6, fasciculata 11.4, reticularis 11.1%), 3, 9 (glomerulosa 16.6, fasciculata 18.0, reticularis 18.0%), reaching the maximum and decreased to day 14, month 1 (glomerulosa 11.4, fasciculata 11.0, reticularis 10.7%) 2 (glomerulosa 8.5, fasciculata 7.8, reticularis 8.8%), month 6 (glomerulosa 4.1, fasciculata 4.2, reticularis 3.8%), 12 and 24 (Fig. 24C). The increase and decrease were stochastically significant ($P < 0.01$).

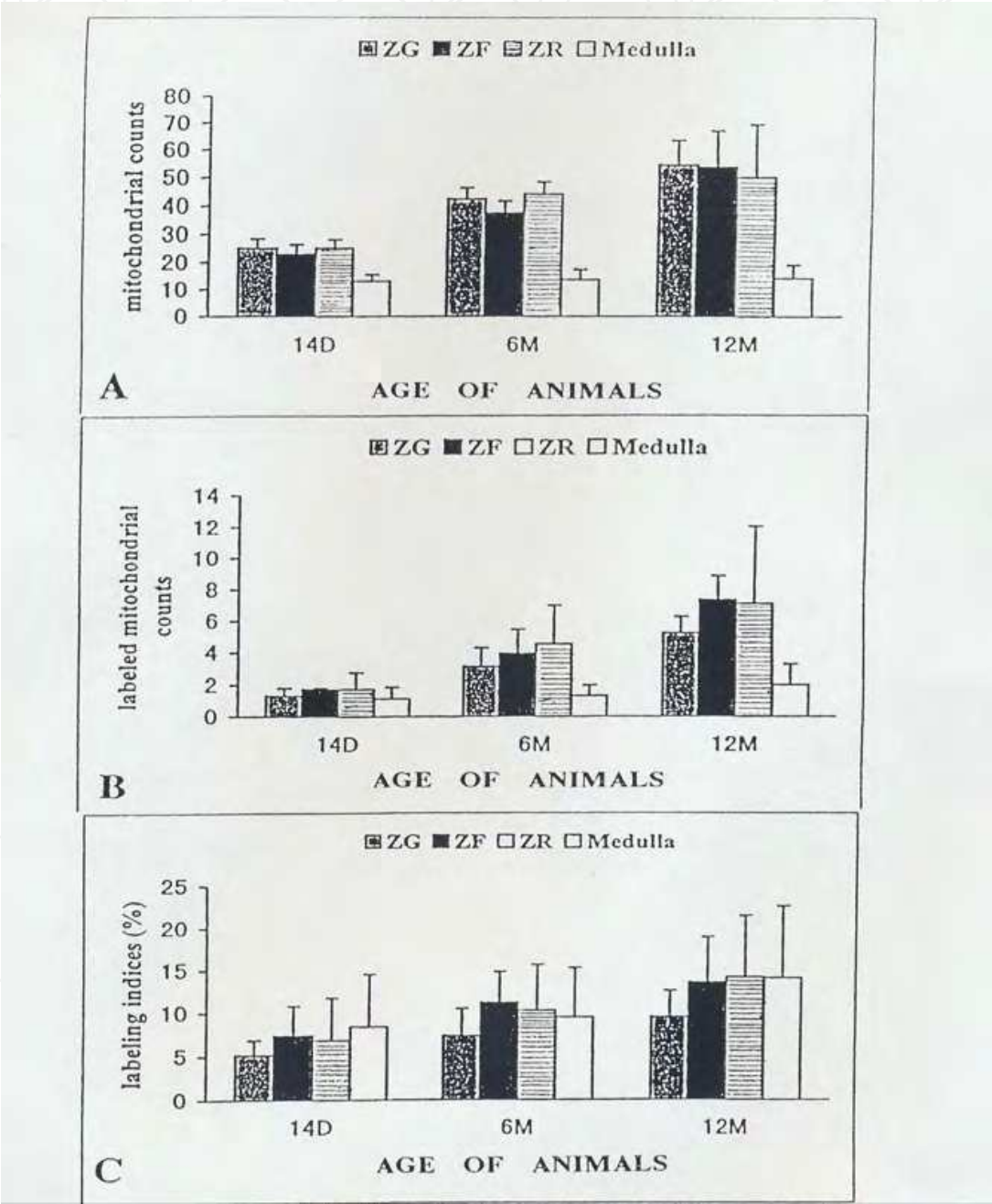


Fig. 24. Histogram showing aging changes of the respective cell types of the adrenal glands of aging mice labeled with ^3H -uridine showing RNA synthesis. Mean \pm Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 173, 2002, Urban & Fischer, Jena, Germany

Fig. 24A. The number of mitochondria per cell.

Fig. 24B. The number of labeled mitochondria per cell.

Fig. 24C. The mitochondrial labeling index.

2.2.2 The RNA synthesis in aging mouse adreno-medullary cells

The adrenal medulla consists of 2 cell types, the adrenalin cells and noradrenalin cells. When they were labeled with ^3H -uridine, an RNA precursor, silver grains appeared over almost all the cells, both nuclei and cytoplasm containing mitochondria (Liang et al. 1999, Nagata et al. 2000b, 2010b). Quantitative analysis revealed that the numbers of mitochondria in both adrenalin and noradrenalin cells at various ages increased from fetal day 19 to postnatal month 1 due to aging of animals, respectively, but did not decrease to month 24 (Fig. 24A), while the number of labeled mitochondria (Fig. 24B) and the labeling indices of intramitochondrial RNA synthesis incorporating ^3H -uridine increased from fetal day 19 to postnatal month 1, reaching the maxima, but did not decrease to month 24 (Fig. 24C). It was shown that the activity of intramitochondrial RNA synthesis in the adrenal medullary cells in aging mice increased but did not decrease due to aging of animals in contrast to DNA synthesis (Nagata 2010b).

2.2.3 The RNA synthesis in the Leydig cells of the testis

The cells of Leydig can be found in the interstitial tissues between the seminiferous tubules of the testis of mammals (Gao 1993, Gao et al. 1994, 1995a, Nagata et al. 2000b). They are identified as spherical, oval, or irregular in shape and their cytoplasm contains lipid droplets. We studied the macromolecular synthesis of the cells in the testis of several groups of litter ddY mice at various ages from fetal day 19 to postnatal aging stages up to 2 years in senescence by LM and EMRAG using ^3H -thymidine, ^3H -uridine and ^3H -leucine incorporations.

The incorporation of ^3H -uridine into RNA was observed in almost all the Leydig cells in the interstitial tissues of the testis from embryonic day 19 to 2 years after birth. A few silver grains over the nuclei and cytoplasm of the Leydig cells labeled with ^3H -uridine were observed at embryonic day 19. The silver grains over those cells slightly decreased at postnatal day 1, 3 (Fig. 21H), 7 and 14. The number of the silver grains over the nuclei increased from postnatal 1 months onwards. The average number of silver grains over the cytoplasm increased gradually and reached the maximum at 12 months after birth. At each stage, the activity of RNA synthesis was specifically localized over the euchromatin in the nucleus and nucleolus as observed by EMRAG. From adult to senescent stages, the activity of RNA synthesis maintained a high level in their nuclei as compared to the cytoplasm. In the cytoplasm of Leydig cells in respective aging groups some of the mitochondria and endoplasmic reticulum were also labeled with silver grains. It is noteworthy that the average grain counts increased prominently in the senescent aging groups at 1 and 2 years after birth.

2.3 The protein synthesis in the endocrine system

We studied the protein synthesis of the adrenal glands and the cells of Leydig in the testis of aging mice among the endocrine organs after the administration of ^3H -leucine at various ages.

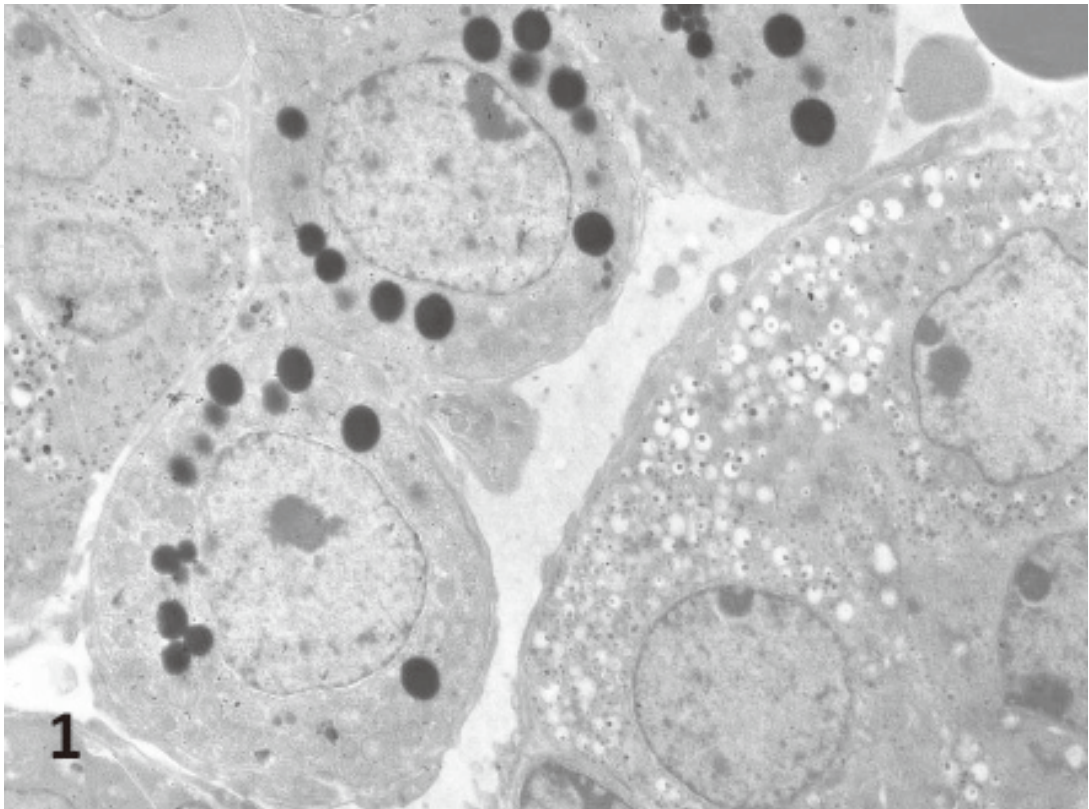
2.3.1 The protein synthesis in the adrenal gland

Observing both LM and EM RAG labeled with ^3H -leucine, demonstrating protein synthesis, the silver grains were found over the nuclei and cytoplasm of almost all the adreno-cortical and adreno-medullary cells from perinatal stages to postnatal month 1-24 (Nagata 2010c,d,e,f,g). Those labeled cells were found in all the 3 layers, the zona glomerulosa, the zona fasciculata and the zona reticularis, as well as in all the cells in the adreno-medullae at respective aging stages.

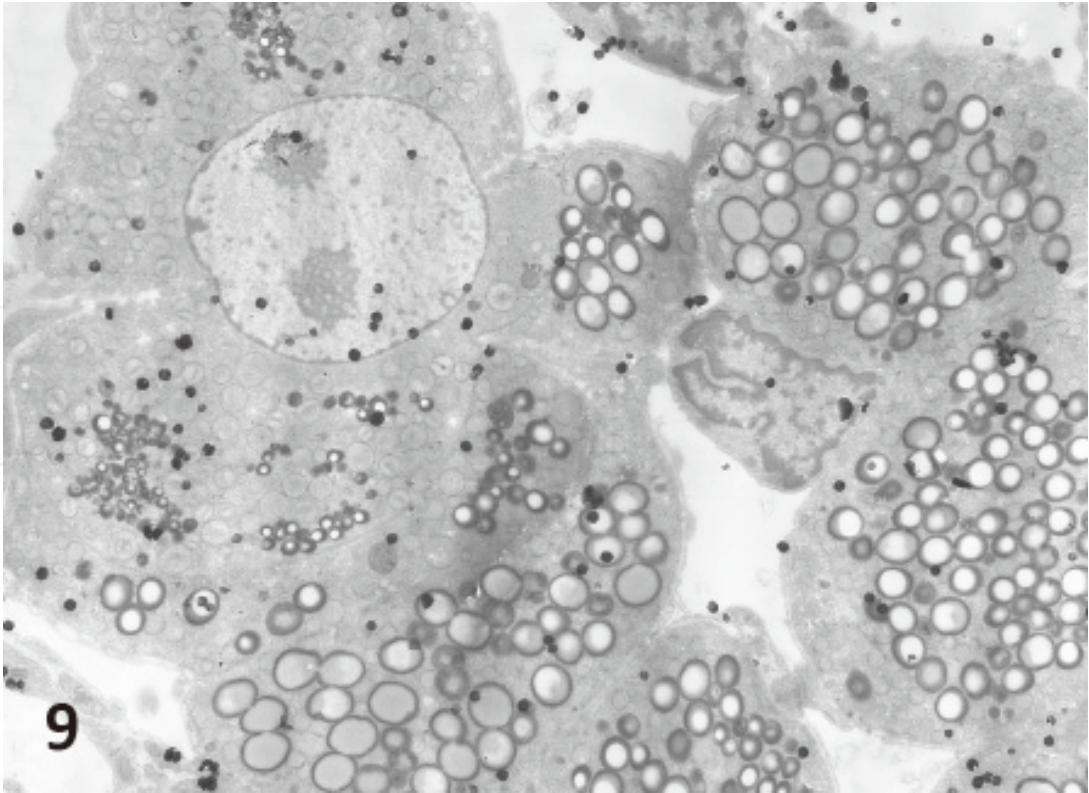
2.3.1.1 Protein synthesis in mitochondria of mouse adrenal cells

In order to study the aging changes of intramitochondrial protein synthesis of mouse adrenal cells, 10 groups of developing and aging mice, each consisting of 3 individuals, total 30, from fetal day 19 to postnatal newborn at day 1, 3, 9, 14, adult at month 1, 2, 6 and senescent animals at month 12 (year 1) and 24 (year 2) were injected with ^3H -leucine, an protein precursor, sacrificed 1 hr later and the adrenal tissues were fixed and processed for electron microscopic radioautography. On electron microscopic radioautograms obtained from each animal, the number of mitochondria per cell, the number of labeled mitochondria with ^3H -leucine showing protein synthesis per cell and the mitochondrial labeling index in each adreno-cortical cells, in 3 zones, as well as in each adreno-medullary cells, 2 types of cells in the medulla, the adrenalin cells and the noradrenalin cells, were calculated and the results in respective aging groups were compared with each others (Nagata 2010c,d,e,f,g). Preliminary quantitative analysis on the number of mitochondria in either 10 adreno-cortical cells or adreno-medullary cells whose nuclei and cytoplasm were labeled with silver grains and other 10 cells whose nuclei and cytoplasm 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 calculated regardless whether their nuclei were labeled or not (Nagata 2010d,e,f,g). The results demonstrated that the number of mitochondria in adreno-cortical cells in 3 zones, the zona glomerulosa, fasciculata and reticularis of respective mice at various ages increased from fetal day 19 to postnatal month 1 reaching the plateau from month 1 to 24 due to development and aging of animals, respectively, while the number of labeled mitochondria per cell and the labeling index of intramitochondrial protein synthesis incorporating ^3H -leucine increased from fetal day 19 to postnatal day 3 to month 2 and decreased to month 24. We carried out the quantitative analysis of these incorporations into nuclei and cell organelles of adrenal cells, both adrenal cortical cells and medullary cells, in aging mice from prenatal to postnatal newborn, juvenile, adult and senescent individuals (Nagata 2010a,b).

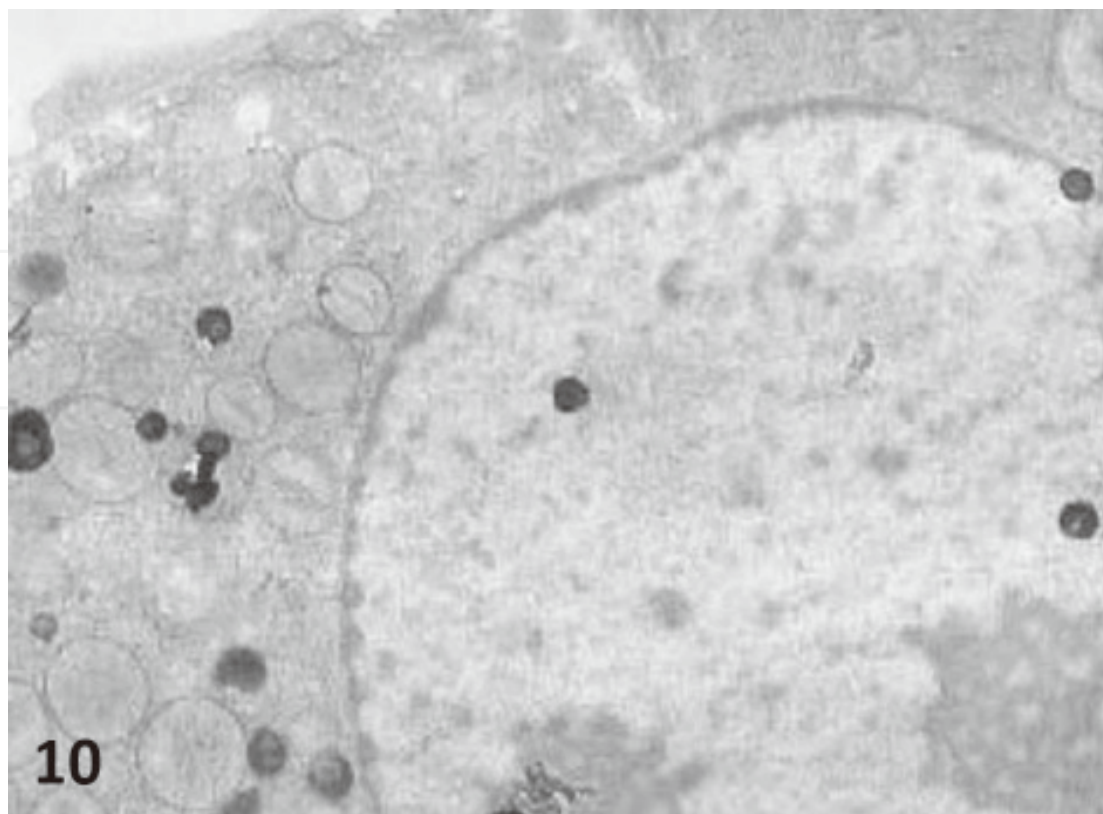
Observing EM radioautograms, the silver grains were found over the nuclei of some adreno-cortical cells labeled with ^3H -leucine demonstrating protein synthesis in all aging stages from perinatal stages at embryonic day 19, postnatal day 1 and day 3, day 9 and day 14 and adults at month 1, month 2, month 6, month 12 and month 24. Those labeled cells were found in all the 3 layers, the zona glomerulosa (Fig. 25A), the zona fasciculata and the zona reticularis (Fig. 25B, C), at respective aging stages. In the labeled adreno-cortical cells in 3 layers the silver grains were mainly localized over the euchromatin of the nuclei or a few or several silver grains were found over cytoplasmic organelles, especially over some of the mitochondria showing protein synthesis incorporating ^3H -leucine. The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices and some over the mitochondrial membranes as observed by high power magnification (Fig. 25C).



25A



25B



25C

Fig. 25. EMRAG of the adrenal cortical cells aging mice labeled with ^3H -leucine showing protein synthesis in the nucleus as well as in a few mitochondria. From Nagata, T.: *Annals of Microscopy* Vol. 4, p. 54-71, 2011.

Fig. 25A. EMRAG of the zona glomerulosa of a juvenile mouse at postnatal day 14, labeled with ^3H -leucine showing protein synthesis (several silver grains) in the nucleus as well as in a few mitochondria. $\times 3,000$.

Fig. 25B. EMRAG of the zona reticularis of an old adult mouse aged at postnatal month 12, labeled with ^3H -leucine showing protein synthesis in the nucleus and a few mitochondria. $\times 3,000$

Fig. 25C. High power magnification EMRAG of Fig. 24B, the zona reticularis of an old adult mouse aged at postnatal month 12, showing protein synthesis in a few mitochondria at upper left corner. $\times 15,000$.

2.3.1.1.1 Number of mitochondria of adreno-cortical cells in aging mice labeled with ^3H -leucine

Preliminary quantitative analysis on the number of mitochondria in either 10 adreno-cortical cells whose nuclei and cytoplasm were labeled with ^3H -leucine showing 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 calculated regardless whether their nuclei were labeled or not (Fig. 26A). 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 13.5, fasciculata 14.9, reticularis 15.2/cell) to postnatal day 14 (glomerulosa 37.7, fasciculata 37.8, reticularis 39.8/cell), and to adult stages at postnatal month 1 (glomerulosa 41.5, fasciculata 42.3, reticularis 42.9/cell), then increased at month 2 (glomerulosa 64.2, fasciculata 65.1, reticularis 67.2/cell), but kept plateau from month 6 (glomerulosa 61.7, fasciculata 62.9, reticularis 62.1/cell), to month 12 (glomerulosa 59.4, fasciculata 70.5, reticularis 71.4/cell) and month 24 (glomerulosa 59.5, fasciculata 62.2, reticularis 63.3/cell). The increase from embryo day 19 to postnatal month 1 was stochastically significant ($P < 0.01$).

2.3.1.1.2 Number of labeled mitochondria of adreno-cortical cells in aging mice labeled with ^3H -leucine

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 ^3H -leucine demonstrating protein synthesis in 10 aging groups at perinatal stages, prenatal embryo day 19, postnatal day 1, 3, 9 and 14, month 1, 3, 6, 12 and 24, showed that the numbers of labeled mitochondria with ^3H -leucine showing protein synthesis per cell gradually increased from prenatal embryo day 19 (glomerulosa 0.3, fasciculata 0.4, reticularis 0.4/cell) to postnatal day 1 (glomerulosa 0.5, fasciculata 0.6, reticularis 0.5/cell), day 3 (glomerulosa 1.2, fasciculata 0.8, reticularis 1.1/cell), day 9 (glomerulosa 0.8, fasciculata 1.1, reticularis 1.1/cell), day 14 (glomerulosa 1.5, fasciculata 1.5, reticularis 1.6/cell), and month 1 (glomerulosa 1.8, fasciculata 1.8, reticularis 2.2/cell) and month 2 (glomerulosa 5.4, fasciculata 5.3, reticularis 5.8/cell), reaching the maximum, then decreased to month 6 (glomerulosa 4.5, fasciculata 4.8, reticularis 5.1/cell), month 12 (glomerulosa 5.2, fasciculata 5.8, reticularis 6.0/cell) and 24 (glomerulosa 3.8, fasciculata 4.1, reticularis 4.3/cell), as demonstrated in Fig. 26B.

2.3.1.1.3 Labeling index of mitochondria of adrenal cortical cells in aging mice labeled with ^3H -leucine

Finally, the labeling indices of adreno-cortical cells showing protein synthesis in respective aging stages were calculated from the number of labeled mitochondria (Fig. 26B) dividing by the number of total mitochondria per cell (Fig. 26A), which were plotted in Fig. 26C, respectively.

The results showed that the labeling indices gradually increased from prenatal day 19 (glomerulosa 2.2, fasciculata 2.7, reticularis 2.6%) to postnatal newborn stage at postnatal day 1 (glomerulosa 2.2, fasciculata 2.4, reticularis 2.0%) and day 3 (glomerulosa 4.5, fasciculata 2.9, reticularis 3.9%), and to juvenile stage at postnatal day 9 (glomerulosa 2.8, fasciculata 3.7, reticularis 3.7%), day 14 (glomerulosa 3.9, fasciculata 3.9, reticularis 4.0%) and to the adult stage at month 1 (glomerulosa 4.3, fasciculata 4.2, reticularis 5.1%) and month 2 (glomerulosa 8.5, fasciculata 8.1, reticularis 8.6%), reaching the maximum, and decreased to month 6 (glomerulosa 7.3, fasciculata 7.6, reticularis 8.1%) to month 12 (glomerulosa 8.4, fasciculata 8.2, reticularis 8.4%) and finally to senescence at month 24 (glomerulosa 6.1, fasciculata 6.6, reticularis 6.8%), as is shown in the histogram (Fig. 26C).

From the results obtained it was shown that the labeling indices of the adreno-cortical cells in 3 layers of each animal labeled with ^3H -leucine demonstrating protein synthesis in 10 groups gradually increased from prenatal embryo day 19 to postnatal day 1, 3, 9, and 14, month 1, 2, reaching the maximum, and decreased to 6, 12 and 24, due to aging and senescence.

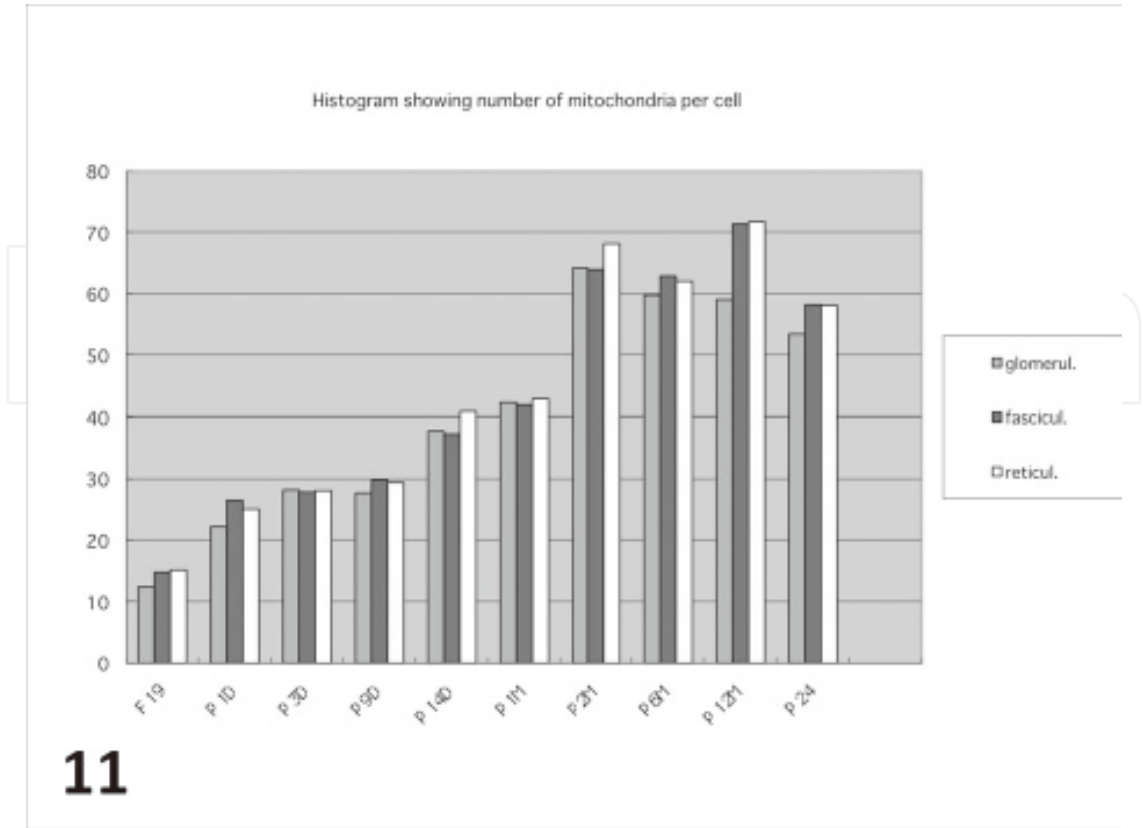


Fig. 26A.

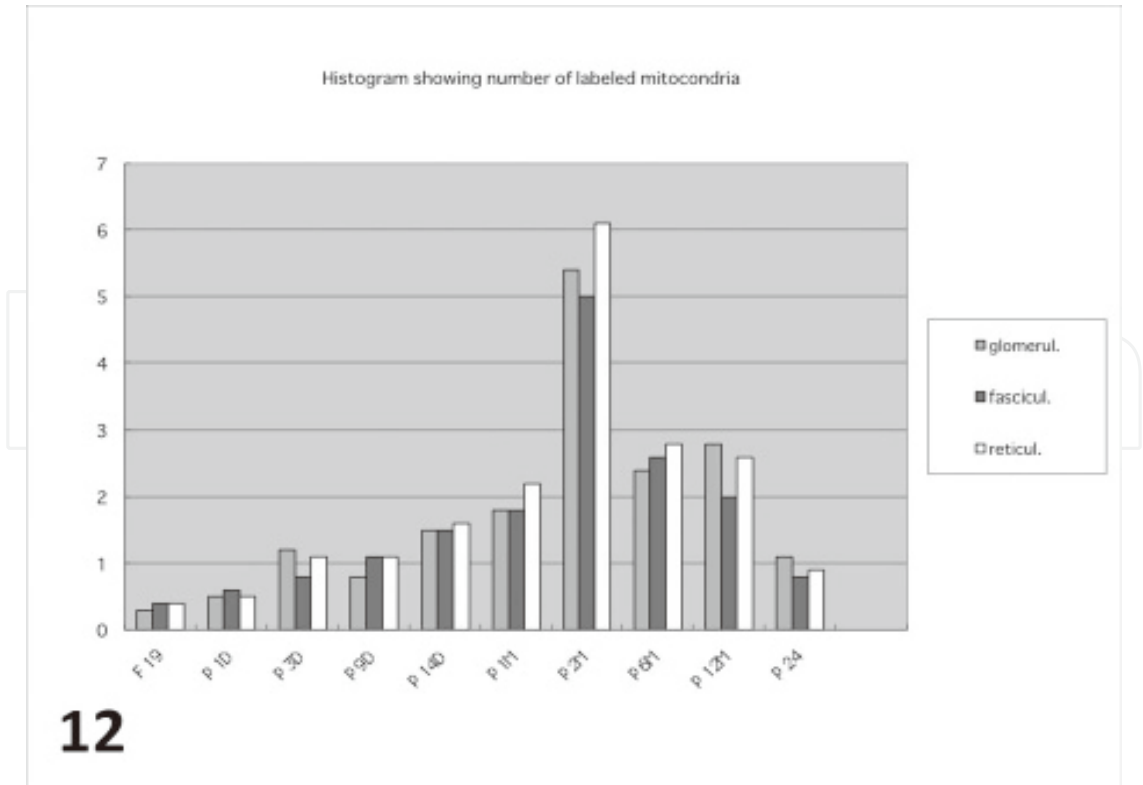
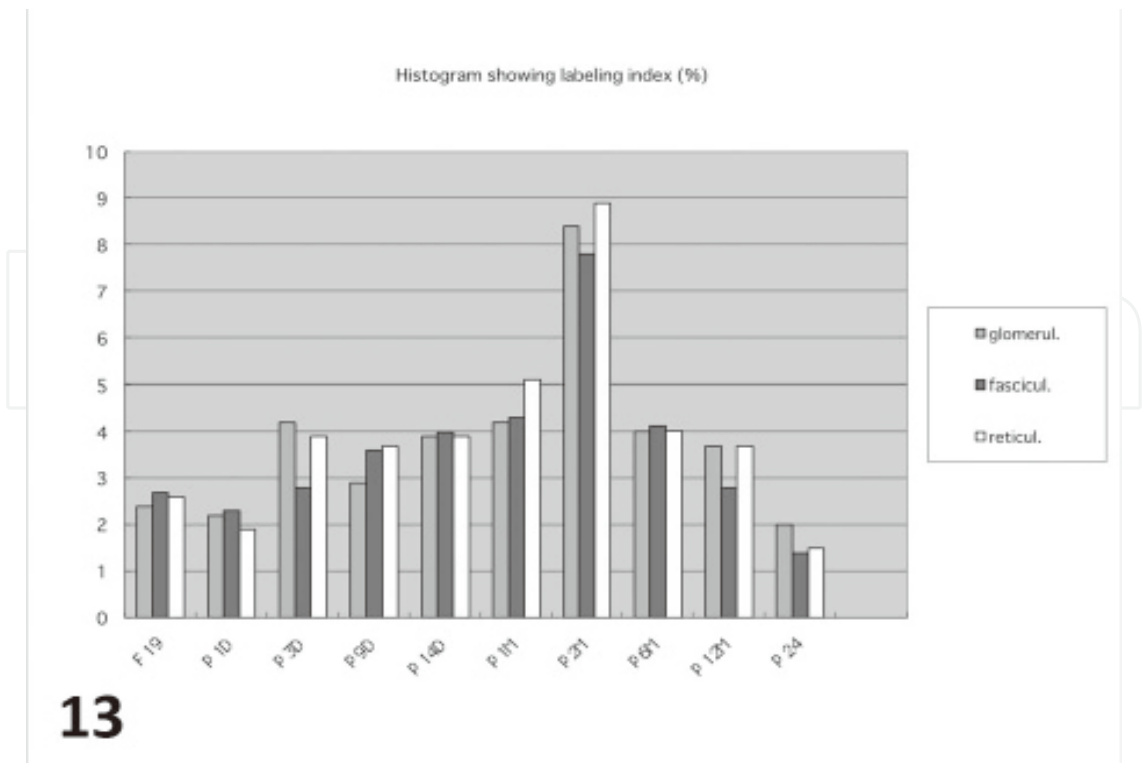


Fig. 26B.



13

Fig. 26C.

Fig. 26. Histogram showing aging changes of the mitochondria in each adreno-cortical cell in the 3 layers of respective animals in 10 aging groups. From Nagata, T.: Annals of Microscopy Vol. 4, p. 54-71, 2011.

Fig. 26A. Histogram showing aging changes of the average numbers of mitochondria per cell in each adreno-cortical cell in the 3 layers of respective animals in 10 aging groups
Fig. 26B. Histogram showing aging changes of the average numbers of labeled mitochondria with ³H-leucine showing protein synthesis per cell in each adrenocortical cell in the 3 layers of respective animals in 10 aging groups.

Fig. 26C. Histogram showing aging changes of the average labeling index of mitochondria labeled with ³H-leucine showing protein synthesis per cell in each adreno-cortical cell in the 3 layers of respective animals in 10 aging groups.

As for the macromolecular synthesis in various cells in various organs of experimental animals observed by light and electron microscopic radioautography, it is well known that the silver grains due to radiolabeled ³H-thymidine demonstrated DNA synthesis, while the grains due to ³H-uridine demonstrated RNA synthesis. On the other hand, the radioautograms showing incorporations of ³H-leucine into mitochondria indicating mitochondrial protein synthesis (Nagata 2002, 2009c,d,e, 2010a,b,c). It was shown from the results that the silver grains localized over the mitochondria independently from the nuclei whether the nuclei were labeled with silver grains or not in almost all the cells in the 3 layers of the adreno-cortical cells from prenatal embryo day 19 to postnatal month 24, administered with ³H-leucine during the development and aging. The numbers of labeled mitochondria showing protein synthesis increased from perinatal day to postnatal adult stage at month 2, then kept plateau, while the labeled mitochondria with ³H-leucine showing protein synthesis increased from perinatal stage to postnatal adult stage at month

2, then decreased at month 24, while the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 9, then decreased from day 14 to senescence at month 24, then decreased to the adult stages at month 1 and 2, to month 6, 12 and 24. These changes demonstrate the chronological aging changes. The results obtained previously (Nagata 2008a,b,c,d, 2009a, Nagata & Ito 1996) indicated that mitochondria in the adreno-cortical cells proliferated from newborn to adult stages around month 1 and 2, showing mitochondrial DNA synthesis, while the mitochondrial RNA synthesis increased from newborn stage to postnatal day 9, then decreased from day 14, reaching the maximum, then decreased to month 24, but the RNA synthetic activity was kept plateau from day 14 to month 12, then decreased to senescent at month 24 due to aging (Nagata 2010e).

2.3.1.2 Protein synthesis in mitochondria of mouse adreno-medullary cells

The adreno-medullary tissues obtained from ddY strain mice at various ages from embryo day 19 to postnatal day 30, consisted mainly of 2 cell types, as observed by electron microscopy, adrenalin cells and noradrenalin cells, developing gradually (Nagata 2010a,b). At embryonic day 19 and postnatal day 1, the adreno-medullary cells were composed mainly of polygonal epitheloid cells, surrounded by blood capillaries and fibroblasts. The medullary cells can be divided into 2 types by the ultrastructure of granules. Some of the medullary cells possessed many granules of medium electron density which were believed to correspond to the adrenalin granules, while some other cells possessed many granules of very high electron density which were believed to correspond to the noradrenalin granules. However, the numbers of mitochondria found in their cytoplasm were not so many. At postnatal day 1, day 3 and day 9, the 2 types of cells differentiated and the numbers of granules, both adrenalin and noradrenalin granules, increased respectively. Likewise, the numbers of mitochondria also increased from prenatal day to postnatal days. At postnatal day 14 to month 1, month 2, month 6, month 12 and month 24 the numbers of adrenalin and noradrenalin granules as well as mitochondria increased. At postnatal month 1 and 2, the ultrastructures of 2 cell types were completely developed and the arrangements of the cells in the medulla became typical as adult tissues. Thus, the ultrastructure of the adrenal medullary cells showed changes due to development and aging at respective developmental stages. The number of mitochondria per cell increased from perinatal stage to postnatal stages due to aging. The data were stochastically analyzed using variance and Student's t-test. The increases of mitochondrial numbers in both adrenalin and noradrenalin cells from embryonic day 19 to postnatal month 6 were considered to be significant at P value <0.01.

Observing electron microscopic radioautograms, the silver grains were found over the nuclei of some adreno-medullary cells labeled with ^3H -leucine, demonstrating protein synthesis less in perinatal stages at embryonic day 19, postnatal day 1, day 3, day 9, while more day 14 and adults at month 1, month 2, month 6, month 12 and month 24.

However, those labeled cells were found in all the 2 cell types, adrenalin cells and noradrenalin cells, at respective aging stages. In the labeled adreno-medullary cells 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 adrenalin cells. Likewise, most noradrenalin cells were also labeled with several silver grains in their nuclei as well as in their cytoplasm not only over the mitochondria but also over ribosomes, showing protein

synthesis due to the incorporations of ^3H -leucine especially at the senescent stages from postnatal month 12 when observed at high power magnifications by high voltage electron microscopy. The localizations of silver grains over the mitochondria were not only on the mitochondrial matrices but also over mitochondrial membranes (Nagata 2010a,b).

2.3.1.2.1 Number of mitochondria of adreno-medullary cells in aging mice labeled with ^3H -leucine

Preliminary quantitative analysis on the number of mitochondria in 10 adreno-medullary 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 calculated in 10 adreno-medullary cells regardless whether their nuclei were labeled or not. The results obtained from the number of mitochondria in adreno-medullary cells of respective animals in 10 aging groups at perinatal stages, from prenatal embryo day 19 to postnatal day 1, 3, 9, 14, and month 1, 2, 6, 12, showed an gradual increase from the prenatal day 19 (adrenalin 17.8, noradrenalin 18.2/cell) to postnatal day 1 (adrenalin 21.5, noradrenalin 22.4/cell), day 3 (adrenalin 22.5, noradrenalin 22.9/cell), day 9 (adrenalin 23.5, noradrenalin 23.9/cell), day 14 (adrenalin 24.1, noradrenalin 24.4/cell), and to adult stages at postnatal month 1 (adrenalin 24.7, noradrenalin 23.9/cell), month 2 (adrenalin 25.1, noradrenalin 24.5/cell) and month 6 (adrenalin 24.8, noradrenalin 24.3/cell), month 12 (adrenalin 24.5, noradrenalin 24.1/cell), and month 24 (adrenalin 23.5, noradrenalin 23.3/cell). The increases of mitochondrial numbers in both adrenalin and noradrenalin cells from embryonic day 19 to postnatal month 2 were considered to be significant at P value < 0.01 . However, the slight decrease from month 6 to 24 was not significant (Nagata 2010a or b).

2.3.1.2.2 Number of labeled mitochondria of adrenal medullary cells in aging mice labeled with ^3H -leucine

We counted the number of mitochondria labeled with silver grains obtained from 10 adreno-medullary cells in the 3 layers of each animal labeled with ^3H -leucine demonstrating protein synthesis in 10 aging groups at perinatal stages, prenatal embryo day 19, postnatal day 1, 3, 9 and 14, month 1, 3, 6, 12 and 24, as well as the number of all the mitochondria and calculated the labeling index.

The results demonstrated that the numbers of labeled mitochondria with ^3H -leucine showing protein synthesis per cell gradually increased from prenatal embryo day 19 (adrenalin 0.5, noradrenalin 0.5/cell), to postnatal day 1 (adrenalin 0.65, noradrenalin 0.6/cell), day 3 (adrenalin 0.7, noradrenalin 0.75/cell), day 9 (adrenalin 0.8, noradrenalin 0.8/cell), day 14 (adrenalin 0.8, noradrenalin 0.9/cell), reaching the maxima at month 1 (adrenalin 0.9, noradrenalin 0.85/cell), and decreased to month 2 (adrenalin 0.8, noradrenalin 0.82/cell), month 6 (adrenalin 0.81, noradrenalin 0.8/cell) month 12 (adrenalin 0.7, noradrenalin 0.75/cell) and month 24 (adrenalin 0.7, noradrenalin 0.65/cell). The data were stochastically analyzed using variance and Student's t -test. The increases of the numbers of labeled mitochondria in both adrenalin and noradrenalin cells from embryo day 19 to postnatal day 14 and month 1, as well as the decreases from month 1 to month 24 were stochastically significant ($P < 0.01$).

2.3.1.2.3 Labeling index of mitochondria of adreno-medullary cells in aging mice labeled with ^3H -leucine

From the results, the labeling indices of mitochondrial protein synthesis in 2 cell types in respective aging stages were calculated from the number of labeled mitochondria dividing by the number of total mitochondria per cell.

The results showed that the labeling indices gradually increased from prenatal day 19 (adrenalin 2.8, noradrenalin 2.6%) to postnatal newborn day 1 (adrenalin 2.8, noradrenalin 2.4%), day 3 (adrenalin 3.3, noradrenalin 2.9%), day 9 (adrenalin 3.4, noradrenalin 3.3%) to juvenile stage at day 14 (adrenalin 3.6, noradrenalin 3.8%), reaching the maximum, and decreased to adult stages at month 1 (adrenalin 3.6, noradrenalin 3.5%), month 2 (adrenalin 3.1, noradrenalin 3.3%), month 6 (adrenalin 3.2, noradrenalin 3.3%), month 12 (adrenalin 2.8, noradrenalin 3.1%) and month 24 (adrenalin 2.9, noradrenalin 2.8%). From the results, the increases of the mitochondrial labeling indices in both the adrenalin and noradrenalin cells from embryo day 19 to postnatal day 14, as well as the decreases from day 14 to month 24 were stochastically significant ($P < 0.01$).

From the results obtained it was shown that intramitochondrial protein synthesis was observed in adreno-medullary cells, both the adrenalin cells and noradrenalin cells of developing and aging mice at various ages from prenatal embryos to postnatal newborn, young juvenile, adult and senescent stages and the number of mitochondria per cell showed increases due to aging, while the number of labeled mitochondria per cell and the labeling indices showed increases and slight decreases due to aging.

As for the macromolecular synthesis in various cells in various organs of experimental animals observed by light and electron microscopic radioautography, it is well known that the silver grains due to radiolabeled ^3H -thymidine demonstrate DNA synthesis, while the grains due to ^3H -uridine demonstrate RNA synthesis and ^3H -leucine protein synthesis (Nagata 1996, 1997, 2002, 2010). We formerly observed the intramitochondrial DNA synthesis as well as RNA synthesis in various cells in mice and rats (Nagata 1972a,b,c,d, 1974, 1984, 2001, 2001, Nagata et al. 1975, 1976, 1977). Lately we observed in the intramitochondrial DNA synthesis as well as RNA synthesis in the adrenal cortical and medullary cells, both the adrenalin cells and the noradrenalin cells at various ages from fetal day 19 to postnatal newborn day 1, 3, 9, juvenile day 14 and to adult month 1, 2, 6, 12 and 24 (Nagata 2008a,b, 2009a, 2010b,e), as well as the intramitochondrial protein synthesis during the aging and senescence (Nagata 2010d,f,g,h).

These results demonstrated that the numbers of silver grains showing nuclear protein synthesis did not give any significant difference between the 2 cells types in the same aging groups from perinatal to senescent stages. The radioautograms showing incorporations of ^3H -leucine into mitochondria indicating mitochondrial protein synthesis resulted in silver grain localization over the mitochondria independently from the nuclei whether the nuclei were labeled with silver grains or not in both cell types, adrenalin and noradrenalin cells, in the medullae from prenatal embryo day 19 to postnatal day 1, 3, 9 and 14, to postnatal month 1, 2, 6, 12 and 24, during the development and aging. The numbers of labeled mitochondria showing protein synthesis as well as the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 14 to month 12, reaching the maxima, and then did not decrease to the senescent stages at month 24 (year 2).

From the results obtained, it was shown that intramitochondrial protein synthesis was observed in adreno-medullary cells, both the adrenalin cells and noradrenalin cells of developing and aging mice at various ages from prenatal embryos to postnatal newborn, young juvenile, adult and senescent stages and the number of mitochondria per cell showed increases due to aging, while the number of labeled mitochondria per cell and the labeling indices showed increases and slight decreases due to aging (Nagata 2010a,b,c,d,e,f,g,h).

As for the macromolecular synthesis in various cells in various organs of experimental animals observed by light and electron microscopic radioautography, it is well known that the silver grains due to radiolabeled ^3H -thymidine demonstrate DNA synthesis, while the grains due to ^3H -uridine demonstrate RNA synthesis and ^3H -leucine protein synthesis (Nagata et al. 1967a, b, Nagata 1972a, b, c, 2001, 2002). The previous results obtained from the studies on the adreno-cortical cells of aging mice by light microscopic radioautography revealed that silver grains indicating DNA synthesis incorporating ^3H -thymidine were observed over the nuclei of some adreno-cortical cells at perinatal stages from postnatal day 1 to day 14 (Ito 1996, Ito & Nagata 1996). However, they did not observe the intramitochondrial RNA synthesis. We formerly observed the intramitochondrial DNA synthesis (Nagata et al. 1967a, b, Nagata 1972a, b, c, 2001, 2002) as well as RNA synthesis (Nagata 1972a,b,c,d, 1974, 1984, 2001, 2002, Nagata et al. 1975, 1976, 1977, Nagata & Murata 1977) in the adrenal medullary cells, both the adrenalin cells and the noradrenalin cells at various ages from fetal day 19 to postnatal newborn day 1, 3, 9, juvenile day 14 and to adult month 1, 2, 6, 12 and 24. In the present study, we also observed the intramitochondrial protein synthesis during the aging and senescence. The numbers of silver grains showing nuclear protein synthesis did not give any significant difference between the 2 cells types in the same aging groups from perinatal to senescent stages. The radioautograms showing incorporations of ^3H -leucine into mitochondria indicating mitochondrial protein synthesis resulted in silver grain localization over the mitochondria independently from the nuclei whether the nuclei were labeled with silver grains or not in both cell types, adrenalin and noradrenalin cells, in the medullae from prenatal embryo day 19 to postnatal day 1, 3, 9 and 14, to postnatal month 1, 2, 6, 12 and 24, during the development and aging. The numbers of labeled mitochondria showing protein synthesis as well as the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 14 to month 12, reaching the maxima, and then did not decrease to the senescent stages at month 24 (year 2).

With regards to DNA in mitochondria in animal cells or plastids in plant cells, many studies have been reported in various cells of various plants and animals since 1960s (Nass 1966, Nass and Nass 1963, Gibor and Granick 1964, Gahan and Chayen 1965). Most of these authors observed DNA fibrils in mitochondria which were histochemically extracted by DNase. Electron microscopic observation of the DNA molecules isolated from the mitochondria revealed that they were circular in shape, with a circumference of 5-6 μm . It was calculated that such a single molecule had a molecular weight of about 10^7 daltons (van Bruggen et al. 1966). Mitochondria of various cells also contained a DNA polymerase, which was supposed to function in the replication of the mitochondrial DNA. On the other hand, the incorporations of ^3H -thymidine into mitochondria demonstrating DNA synthesis were observed by means of electron microscopic radioautography in lower organism such as slime mold (Guttes and Guttes 1964, Schuster 1965), tetrahymena (Stone and Miller Jr.

1965) or chicken fibroblasts in tissue culture under abnormal conditions (Chévremont 1963). However, these authors used old-fashioned developers consisting of methol and hydroquinone (MQ-developer) which produced coarse spiral silver grains resulting in inaccurate localization over cell organelles when observed by electron microscopy. All of these authors showed photographs of electron radioautographs with large spiral-formed silver grains (2-3 μm in diameter) localizing not only over the mitochondria but also outside the mitochondria. In order to obtain smaller silver grains, we first used elon-ascorbic acid developer after gold latensification, which produced comma-shaped smaller silver grains (0.4-0.8 μm in diameter), then later we used phenidon developer after gold latensification, producing dot-like smaller silver grains (0.2-0.4 μm in diameter) localizing only inside the mitochondria showing ultrahigh resolution of radioautograms. These papers were the first which demonstrated intramitochondrial DNA synthesis incorporating ^3H -thymidine with accurate intramitochondrial localization in avian and mammalian cells. With regards the resolution of electron microscopic radioautography, on the other hand, many authors discussed the sizes of silver grains under various conditions and calculated various values of resolutions (Salpeter et al. 1969, Nadler 1971, Uchida & Mizuhira 1971, Nagata 1972b,c). Those authors who used the M-Q developers maintained the resolution to be 100-160 nm (Salpeter et al. 1969, Nadler 1971), while those authors who used the elon-ascorbic acid developer (Nagata 1972b, , Uchida & Mizuhira 1971) calculated it to be 25-50 nm. When we used phenidon developer at 16°C for 1 min after gold latensification, we could produce very fine dot-shaped silver grains and obtained the resolution around 25 nm (Nagata 1992, 1996, 1997, 2001, 2002, Murata et al. 1979). For the analysis of electron radioautographs, Salpeter et al. (1969) proposed to use the half-distance and very complicated calculations through which respective coarse spiral-shaped silver grains were judged to be attributable to the radioactive source in a certain territory within a resolution boundary circle. However, since we used phenidon developer after gold latensification to produce very fine dot-shaped silver grains, we judged only the silver grains which were located in the mitochondria which were dot-shaped very fine ones to be attributable to the mitochondria without any problem as was formerly discussed (Nagata 1972a,b, c, 1996, 1997, 2001, 2002).

Then we also demonstrated intramitochondrial DNA synthesis incorporating ^3H -thymidine in some other established cell lines originated from human being such as HeLa cells (Nagata 1972a,b,c,d) or mitochondrial fractions prepared from in vivo mammalian cells such as rat and mouse (Nagata 1974, Nagata et al. 1975, 1976). It was later commonly found in various cells and tissues not only in vitro obtained from various organs in vivo such as the cultured human HeLa cells (Nagata et al. 1966, 1986, Nagata 1984), cultured rat sarcoma cells (Nagata et al. 1977), mouse liver and pancreas cells in vitro (Nagata & Murata 1977, Nagata et al. 1977, 1986), but also in vivo cells obtained from various organs such as the salivary glands (Nagata et al. 2000), the liver (Nagata 2003, 2006a,b, 2007a,b,c,d,e, Nagata & Ma 2005, Nagata et al. 1979, 1982a,b, Ma & Nagata 1988a,b, Ma et al. 1994), the pancreas (Nagata 1992, Nagata et al. 1986), the trachea (Sun et al. 1997), the lung (Sun et al. 1994, 1995a,b, Nagata 2007), the kidneys (Hanai & Nagata 1994, Nagata 2005), the testis (Gao et al. 1994, 1995), the uterus (Yamada et al. 1993, 1994), the adrenal glands (Ito 1996, Ito et al. 1996, Nagata 2008a,b, 2009g,j, 2010a,b), the brains (Cui et al. 1996), and the retina (Gunarso 1984, Gunarso et al. 1996, 1997, Kong & Nagata 1994, Nagata 1996) of mice, rats and chickens. Thus, it is clear that all the cells in various organs of various animals synthesize DNA not only in their nuclei but also in their mitochondria.

The relationship between the intramitochondrial DNA synthesis and cell cycle was formerly studied in synchronized cells and it was clarified that the intramitochondrial DNA synthesis was performed without nuclear involvement (Nagata 1972b). However, the relationship between the DNA synthesis and the aging of individual animals and men has not yet been clarified except a few papers recently published by Korr and associates on mouse brain (Korr et al. 1997, 1998, Schmitz et al. 1999a,b). They reported both nuclear DNA repair, measured as nuclear unscheduled DNA synthesis, and cytoplasmic DNA synthesis labeled with ^3H -thymidine in several types of cells in brains such as pyramidal cells, Purkinje cells, granular cells, glial cells, endothelial cells, ependymal cells, epithelial cells as observed by light microscopic radioautography using paraffin sections observed by LMRAG. They observed silver grains over cytoplasm of these cells by light microscopy and maintained that it was reasonable to interpret these labeling as ^3H -DNA outside the nuclei, which theoretically belonged to mitochondrial DNA without observing the mitochondria by electron microscopy. From the results, they concluded that distinct types of neuronal cells showed a decline of both unscheduled DNA and mitochondrial DNA syntheses with age in contrast that other cell types, glial and endothelial cells, did not show such age-related changes neither counting the number of mitochondria in respective cells nor counting the labeling indices at respective aging stages. Thus, their results from the statistics obtained from the cytoplasmic grain counting seems to be not accurate without observing mitochondria directly. To the contrary, we had studied DNA synthesis in the livers of aging mice (Nagata et al. 1979, Nagata 1982a,b, 2003, 2005, 2006a,b,c,d,e, 2010c, Ma et al. 1988, 1994, Ma & Nagata 1988) and clearly demonstrated that the number of mitochondria in each hepatocytes, especially mononucleate hepatocytes, increased with the ages of animals from the perinatal stages to adult and senescent stages, while the number of labeled mitochondria and the labeling indices increased from the perinatal stages, reaching a maximum at postnatal day 14, then decreased. We also demonstrated that the number of mitochondria and labeled mitochondria with ^3H -thymidine, ^3H -uridine and ^3H -leucine in the adrenal glands in aging mice increased due to aging (Nagata 2009j, 2010a,b,d,e,f,g,h).

2.3.2 The protein synthesis in the Leydig cells of the testis

We studied the macromolecular synthesis of the cells in the testis of several groups of litter ddY mice at various ages from fetal day 19 to postnatal aging stages up to 2 years senescence by LM and EM RAG using ^3H -thymidine, ^3H -uridine and ^3H -leucine incorporations (Gao 1993, Gao et al. 1994, 1995a, Nagata 2000b). The incorporation of ^3H -leucine into proteins was observed in almost all the Leydig cells in the interstitial tissues of the testis. The silver grains were located over the nuclei and cytoplasm of respective Leydig cells. The aging change of protein synthesis of Leydig cells among different aging groups was also found (Nagata 2001c, 2002). At embryonic day 19, the silver grains of Leydig cells labeled with ^3H -leucine was observed in both nucleus and cytoplasm and there was no obvious difference between the number of silver grains on the cytoplasm and the nucleus. The number of silver grains decreased at postnatal day 1 and then increased at day 3 and 7. However, the number of silver grains on both the nucleus and cytoplasm decreased from 1 month to 3 months and increased again from 6 months onwards maintaining a high level from adult to senescent stages. Some of the silver grains were also localized over some of the mitochondria in respective aging groups as observed by EM RAG. These results indicate that the DNA, RNA and protein syntheses in Leydig cells are maintained at rather high level even at senescent stages at postnatal 1 and 2 years when the animals survived for longer lives.

2.4 The localization of mercury in the thyroid gland

We studied incorporations of mercury chloride into human thyroid tissues of both normal and cancer cells obtained from human patients (Nagata et al. 1977b, Nagata 1994a,b,c,d,e). The tissues were obtained surgically from human patients of both sexes in various ages suffering from the cancer of thyroids and the both normal and cancer cells were cut into small pieces (3x3 mm) aseptically which were incubated in a medium (Eagle's MEM) containing RI-mercury chloride ($^{203}\text{HgCl}_2$) and fixed either cryo-fixation at -196°C and freeze-dried or chemically fixed with buffered glutaraldehyde and osmium tetroxide. The former tissue blocks were processed for dry-mounting radioautography, while the latter were processed for conventional wet-mounting radioautography. The results revealed that the silver grains appeared much more in the cancer cells processed for freeze-fixation and dry-mounting radioautography (Fig. 21A) than the cancer cells processed for chemical fixation and wet-mounting radioautography (Fig. 21B), as well as much more in the cancer cells than the normal cells under the same conditions. On the other hand, PCNA/cycline and both keratin kinase C and vimentin were immunostained in connection to DNA synthetic activity. It was found that PCNA/cycline, keratin kinase C and vimentin antibodies were localized around the filaments in the thyroid cancer cells (Fig. 21C), demonstrating the relation between those antigens and DNA synthetic activity in cell cycle (Shimizu et al. 1993, Nagata 1994b,c, Gao et al. 1994).

3. Macromolecular synthesis in the nervous system

The nervous system consists of the central nervous system and the peripheral nervous system. The former is divided into the brains and the spinal cord, while the latter into the cerebrospinal system and the autonomous system. We studied macromolecular synthesis of the brains, the spinal cord in the cerebrospinal system and the autonomic peripheral nerves in the autonomous system by LM and EM RAG.

3.1 The DNA synthesis in the nervous system

We studied the DNA synthesis of the brains, the spinal cord in the cerebrospinal system and the autonomic peripheral nerves in the autonomous system by LM and EM RAG (Cui 1995, Cui et al. 1996, Izumiyama et al. 1987, Nagata 1965, 1967a, Nagata and Stegerda 1963, 1964, Nagata et al. 1999a).

3.1.1 The DNA synthesis in the brains

The brains of mammals consist of the cerebrum, the cerebellum and the brain stem. We studied on DNA synthesis and protein synthesis in the cerebellum of aging mouse (Cui 1995, Cui et al. 1996) as well as the glucose incorporation in the cerebrum of adult gerbils (Izumiyama et al. 1987). The DNA synthesis was examined in the cerebella of 9 groups of aging ddY strain mice from fetal day 19, to postnatal day 1, 3, 8, 14 and month 1, 2, 6, 12, each consisting of 3 litter animals, using ^3H -thymidine, a DNA precursor, by LM and EM RAG (Cui 1995, Cui et al. 1996). The labeled nuclei, by the precursor, in both the neurons and glia, i.e., neuroblasts and glioblasts, were observed in the external granular layers of the cerebella of perinatal mice from embryonic day 19 (Fig. 27A) to postnatal day 1, 3, 7 and day 14 by LMRAG and EMRAG. The labeled nuclei disappeared at postnatal 1 month. The peak of labeling index was at postnatal day 3 in both neuroblasts and glioblasts (Fig. 28A, B). The

glioblasts of the external granular layer migrated inward, some of them formed the Bergmann glia cells located between Purkinje cells. Labeled nuclei of neuroblasts and glioblasts in the internal granular layers were observed at perinatal stages. The maximum of the labeling index in the internal granular layer was at postnatal day 3, similarly to the external granular layer. The endothelial cells of the cerebellar vessels were progressively labeled from embryos to neonates, reaching the peak at 1 week after birth and decreasing thereafter.

3.1.2 The DNA synthesis in the peripheral nerves

We first studied the degeneration and regeneration of autonomous nerve cells in the plexuses of Auerbach and Meissner of the jejunums of 15 dogs which were operated upon to produce experimental ischemia of the jejunal loops by perfusing with Tyrode's solution via the mesenteric arteries for 1, 2, 3 and 4 hours (Nagata 1965, 1967, Nagata and Steggerda 1963, 1964). Tissue blocks were obtained from the deganglionated portions and the adjoining normal portions, which were fixed in Carnoy's fluid, embedded in paraffin, sectioned and stained with buffered thionine, methyl-green and pyronine and PAS. Some animals were injected with either ^3H -thymidine or ^3H -cytidine and the intestinal tissues obtained from ischemic portions and normal portions were processed for LM RAG. The results revealed that the ganglion cells in Auerbach's plexus showed various degenerative changes in accordance with the duration of ischemia. After 4 hours ischemia, most of the ganglion cells in Auerbach's plexus were completely destroyed. The degenerative changes in Auerbach's plexus after 4 hours ischemia were irreversible after 1 week recovery. The ganglion cells in the Meissner's plexus, on the other hand, were less sensitive to the ischemia. They recovered completely even after 4 hours ischemia. The PAS positive substances in degenerative ganglion cells in both plexuses decreased immediately after 4 hours ischemia. The DNA contents of ganglion cells in both Auerbach's and Meissner's plexus did not show any change before and after ischemia. The RNA contents decreased immediately after the ischemia (Nagata and Steggerda 1963, 1964). The number of binucleate cells in ganglion cells in both Auerbach's and Meissner's plexuses after 4 hours ischemia increased to 4.6% and 5.7% respectively. In contrast, in the non-ischemic normal control preparations, the binucleate cells occurred only 0.5% and 1.8% in Auerbach's and Meissner's plexus respectively. The high frequency of binucleate cells in the ganglion cells persisted for more than 100 days after the ischemia, indicating a possible regeneration of ganglion cells. The radioautographic study revealed that there was no evidence for DNA synthesis in both Auerbach's and Meissner's plexus from either ischemic or normal loops. The RNA synthesis was observed to be higher in ganglion cells in normal loop than ischemic loop and higher in Auerbach's than in Meissner's as expressed by grain counting. It was higher in binucleate ganglion cells than in mononucleate cells.

3.2 The RNA synthesis in the nervous system

We studied only messenger RNA in the spinal cords of aging mice from perinatal to postnatal adult stages by means of in situ hybridization.

3.2.1 The RNA synthesis in the spinal cord

The localization of TGF- β 1 mRNA in the segments of the spinal cords of mice was investigated by means of in situ hybridization techniques together with immunohistochemical staining (Nagata et al. 1999). The tissues of lower cervical segments of the spinal cords of BALB/c mice,

from embryonic day 12, 14, 16, 19 and postnatal day 1, 3, 7, 14, 21, 28, 42 and 70, were used. For in situ hybridization, ^{35}S -labeled oligonucleotide probes for TGF- β 1 were used to detect their messenger RNA. Cryosections were incubated under silicon cover slides with 100 μl of pre-incubation solution plus final concentration of 2.4×10^6 cpm/ml probes and 100 mM DTT for 16 hours. After washing with SSC and DTT, the slides were dried and processed for radioautography by dipping in Konica NR-M2 emulsion, which were exposed and developed. The results showed that TGF- β 1 mRNA was detectable in the meninges surrounding the spinal cord, but scarcely detected in spinal cord parenchyma (Fig. 27B). The localization of TGF- β 1 mRNA in the spinal cord suggested that TGF- β 1 acted through paracrine mechanism in the morphogenesis of the spinal cord in mice. The localization of TGF- β 1 and its mRNA in the segments of the spinal cords of mice was also investigated with immunocytochemical techniques (Nagata et al. 1999). The tissues of lower cervical segments of the spinal cords of BALB/c mice, from embryonic day 12, 14, 16, 19 and postnatal day 1, 3, 7, 14, 21, 28, 42 and 70, the same as in situ hybridization were used. For immunocytochemistry, transverse cryosections of the spinal cords were cut and stained with rabbit anti-TGF- β 1 polyclonal antibody followed with ABC method. The results showed that positive immunoreactivities arose in the ventral horn motoneurons from the embryonic stage to postnatal neonates (Fig. 27B) up to the adults. The extracellular matrix of the white matter, however, showed positive immunocytochemical staining from postnatal day 14, and thereafter, and the immunoreactivity remained with aging. The whole white matter showed only background level of staining before postnatal day 14. The results indicated that TGF- β 1 regulates motoneuron growth and differentiation as well as they were probably correlated with formation, differentiation and regeneration of myelin of nerve tracts. The immunostaining with β FGF antibody presented the same basal pattern as shown in TGF β 1 immunohistochemistry (Nagata and Kong 1988). The positive immunoreactivities were detected in ganglion cell layer, inner and outer plexiform layers, retinal pigment epithelial layer, choroidal and scleral layers. Since TGF- β 1 mRNA was detectable in the meninges surrounding the spinal cord by in situ hybridization but scarcely detected in spinal cord parenchyma, the disparate localization of TGF- β 1 polypeptide and TGF- β 1 mRNA in the spinal cord suggest that TGF- β 1 acts through paracrine mechanism in the morphogenesis of the spinal cord in mice. The negative control abolished virtually all reactivity when using the normal rabbit serum instead of primary antibody or using avidin-biotin-peroxidase complex solution only.

3.3 The protein synthesis in the cerebellum

When 10 groups of aging ddY mice from fetal day 19, to postnatal day 1, 3, 7, 14 and month 1, 2, 6, 12 and 24, each consisting of 3 litter mates, using ^3H -leucine, protein syntheses of both neuroblasts and glioblasts were observed by LM and EM RAG in the extragranular layers of perinatal animals (Cui 1995, Cui et al. 1996, 2000, Nagata et al. 2001). The silver grains due to ^3H -leucine demonstrating protein synthesis were localized over the nuclei and cytoplasm of neuroblasts and glioblasts of embryos at fetal day 19 and the number of silver grains increased after birth from postnatal day 1, 3 to day 7 and onward. On day 3, some Purkinje cells were recognized incorporating silver grains. The number of silver grains in these cells increased from neonatal stages to mature adult stage at postnatal day 14 and 30, then decreased from month 1, 2, 6, 12 to 24. The increase and decrease of the silver grains were due to the aging changes of protein synthesis in the cerebella due to development and senescence of individual animals.

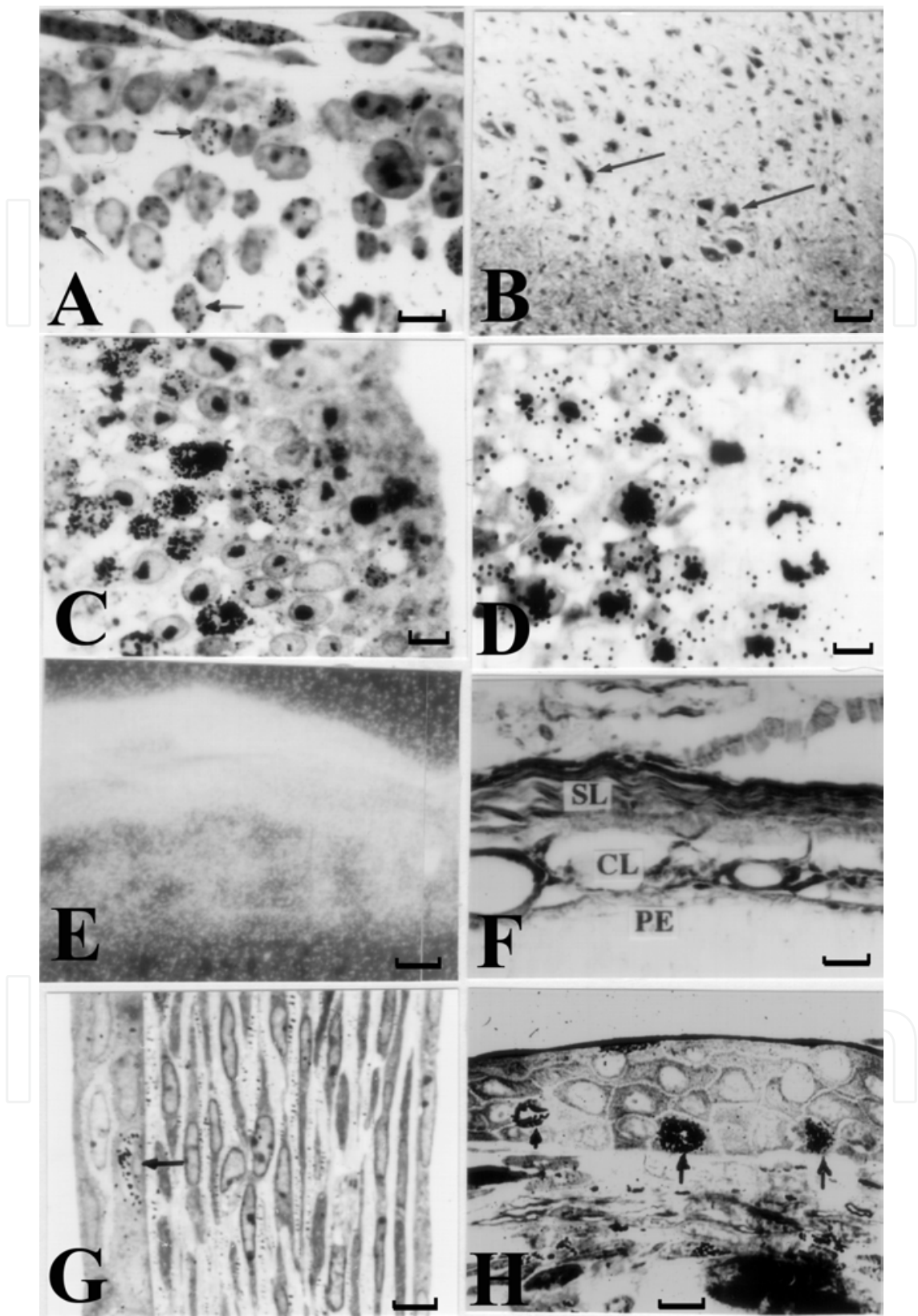


Fig. 27. LM RAG of the neuro-sensory cells. From Nagata, T., *Special Cytochemistry in Cell Biology*, In, *Internat. Rev. Cytol.* Jeon, K. W. Ed., Academic Press, San Diego, USA, London, UK, Vol. 211, No. 1, p. 122, 2001.

Fig. 27A. LM RAG of a prenatal day 19 mouse cerebellum labeled with ^3H -thymidine, showing DNA synthesis. x900.

Fig. 27B. LM RAG of the spinal cord of a postnatal day 14 mouse immunostained with rabbit anti-TGF- β 1 polyclonal IgG followed by ABC method, showing that the ventral horn motoneurons are strongly positive. x70.

Fig. 27C. LM RAG of the optic vesicle of a day 2 chick embryo labeled with ^3H -thymidine, showing DNA synthesis. x750.

Fig. 27D. LM RAG of the optic vesicle of a day 2 chick embryo labeled with ^3H -uridine, showing RNA synthesis. x750.

Fig. 27E. Dark-field LM photo of the scleral layer (top), choroid layer (middle) and pigment epithelium (bottom) of an adult 1 month old mouse demonstrating intense silver grains by in situ hybridization for TGF- β 1 mRNA. x450.

Fig. 27F. Bright-field LM photo of the scleral layer (top), choroid layer (middle) and pigment epithelium (bottom) of an adult 1 month old mouse demonstrating intense silver grains by in situ hybridization for TGF- β 1 mRNA. x450.

Fig. 27G. LM RAG of the cornea of a postnatal day 14 mouse labeled with ^3H -thymidine, showing DNA synthesis in the epithelial nucleus (arrow) as well as in the stroma. x900.

Fig. 27H. LM RAG of the skin of the fore-limb of a salamander at 6 weeks after hatching labeled with ^3H -thymidine, showing DNA synthesis. x900.

3.4 The glucide synthesis in the brains

The incorporation of ^3H -deoxyglucose was studied in the adult gerbil brains among the nervous system of experimental animals (Izumiyama et al. 1987). The changes of soluble deoxyglucose uptake in the hippocampus were studied after ^3H -deoxyglucose injections by means of cryo-fixation, freeze-substitution and dry-mounting radioautography to demonstrate soluble compounds under normal and post-ischemic conditions. The results demonstrated that the neurons in the hippocampus subjected to ischemia revealed higher uptake of soluble glucose than normal control. The concentration of soluble ^3H -deoxyglucose was higher than the chemically fixed and wet-mounted radioautograms that demonstrated only insoluble compounds. However, the relation of glycogen synthesis to aging has not yet been fully clarified.

4. Macromolecular synthesis in the sensory system

The sensory system consists of five organs, i.e., the visual organ or the eye, the stato-acoustic organ or the ear, the gustatory organ or the tongue, the olfactory organ or the nose, and the dermis or the skin. Among these sensory organs, we mainly studied the visual organ and the skin (Gunarso 1983a,b, Gunarso et al. 1996, 1997, Gao et al. 1992a,b, 1993, Kong 1993, Kong and Nagata 1994, Kong et al. 1992a,b, Nagata 1998, 1999, 2000, Nagata et al. 1994, Toriyama 1995).

4.1 The DNA synthesis in the sensory organs

Among the sensory organs, we mainly studied the DNA synthesis of the visual organ and the skin. They should be described separately.

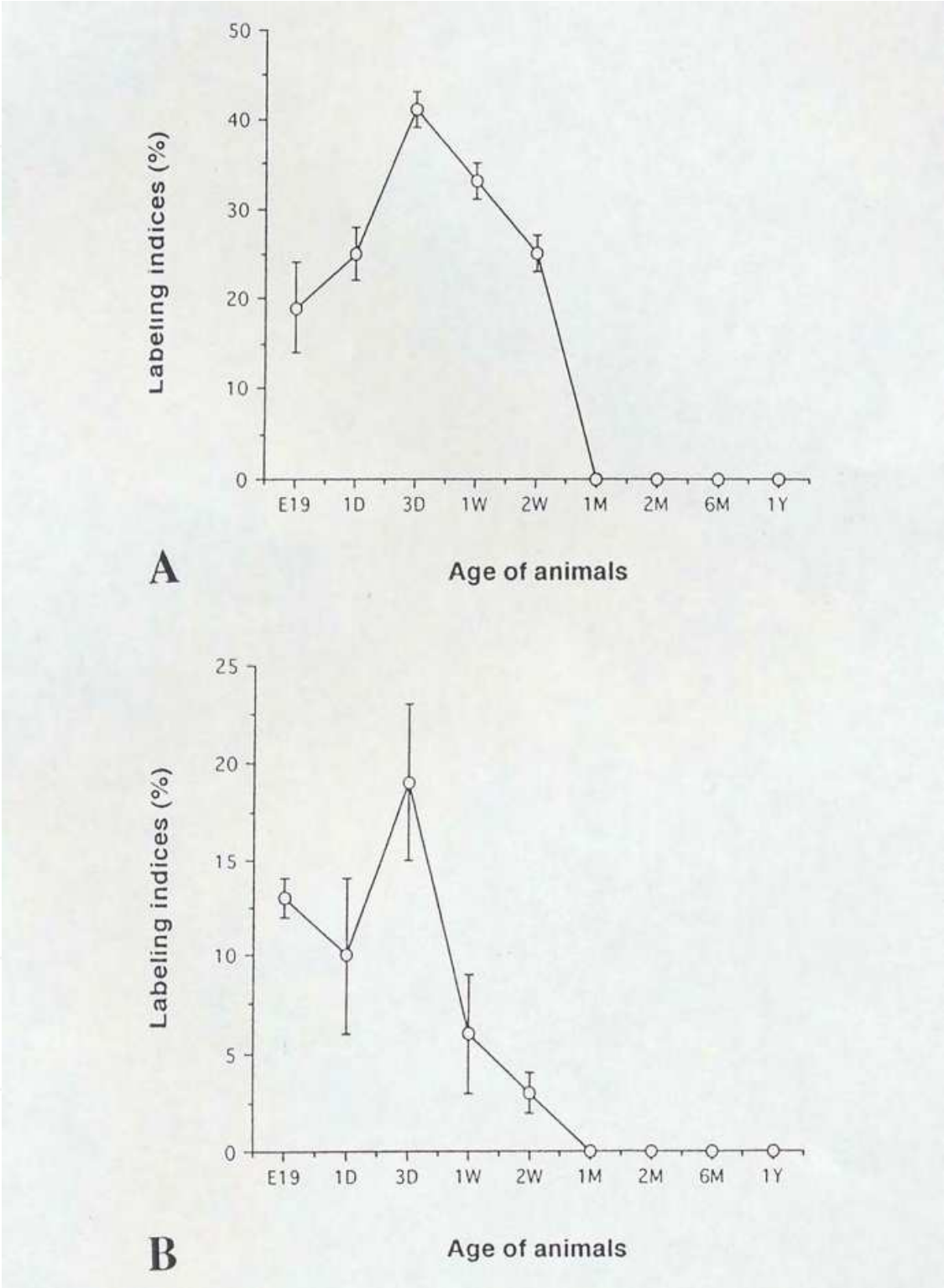


Fig. 28. Transitional curves of the labeling indices of respective cell types in the cerebella of aging mice labeled with ³H-thymidine, showing DNA synthesis. Mean ± Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 186, 2002, Urban & Fischer, Jena, Germany

Fig. 28A. The neuroblasts in the extragranular layer of the cerebella.

Fig. 28B. The glioblasts in the extragranular layer of the cerebella.

4.1.1 The DNA synthesis in the visual organ

The visual organ consists of the eye and its accessory organs. The eye of mammals consists of the cornea, iris, ciliary body, lens, retina, choroid and sclera. We studied mainly the macromolecular synthesis in the retina of chickens and mice (Nagata 2000f). The nucleic acid syntheses, both DNA and RNA, were first studied in the ocular tissues of white Leghorn chick embryos from day 1 to day 14 incubations by LM and EMRAG (Gunarso 1984a,b, Gunarso et al. 1997, Gao et al. 1992a,b, 1993, Kong 1993, Kong and Nagata 1994, Kong et al. 1992a,b, Nagata et al. 1994). It was shown that the labeled cells with silver grains due to ^3H -thymidine were most frequently observed in the nuclei of the retinal cells in the posterior region of the day 2 chick embryo optic vesicle (Fig. 27C) and the labeled cells moved from anterior to posterior regions due to aging by incubation in vitro. The number of labeled cells as expressed by labeling index (%), was more in the posterior regions than the anterior and the equatorial regions and more in the outer portions than in the inner portions at day 2, but the labeling index became more in the anterior regions than the equatorial and posterior regions at day 3, 4 and 7 and it became more in the inner portions than in the outer portions at day 7, decreasing from day 2 to 3, 4 and 7 in each regions (Fig. 28). On the other hand, the silver grains due to ^3H -uridine were observed over the nuclei and cytoplasm of all retinal cells from day 2 to 7 (Fig. 27D) and the number of silver grains incorporating ^3H -uridine increased from day 1 to day 7 and it was more in the anterior regions than in the posterior regions at the same stage (Gunarso et al. 1996). On the other hand, DNA and RNA syntheses in the ocular tissues of aging ddY mice were also studied (Gao et al. 1993, Kong and Nagata 1994, Kong et al. 1992a,b). The ocular tissues taken out from several groups of litter ddY mice at ages varying from fetal day 9, 12, 14, 16, 19 to postnatal day 1, 3, 7, 14 were labeled with ^3H -thymidine in vitro and radioautographed (Gao et al. 1992a,b, 1993, Kong 1993, Kong et al. 1992a,b, Toriyama 1995). Silver grains showing DNA synthesis were localized over the nuclei of retinal cells and pigment epithelial cells in the anterior, equatorial and posterior regions of perinatal animals (Fig. 27A). The labeling indices of the retina and pigment epithelium were higher in earlier stages than in later stages, during which they steadily declined (Fig. 28A,B). However, the retina and the pigment epithelium followed different courses in their changes of labeling indices during embryonic development. In the retina, the labeling indices in the vitreal portions were more than those in the scleral portions during the earlier stages. However, the indices of scleral portions were more than those in the vitreal portions in the later stages. Comparing the three regions of the retinae of mice, the anterior, equatorial and posterior regions, the labeling indices of the anterior region were generally higher than those of the equatorial and posterior regions (Fig. 28A). In the pigment epithelium (Fig. 28B), the labeling indices gradually increased in the anterior region, but decreased in the equatorial and the posterior regions through all developmental stages. These results suggest that the proliferation of both the retina and pigment epithelium in the central region occurred earlier than those of the peripheral regions (Nagata 1999a, Gao et al. 1992a,b, Kong 1993, Kong and Nagata 1994, Kong et al. 1992a, b). In the juvenile and adult stages, however, the labeled cells were localized at the middle of the bipolar-photoreceptor layer of the retina, where was supposed to be the undifferentiated zone.

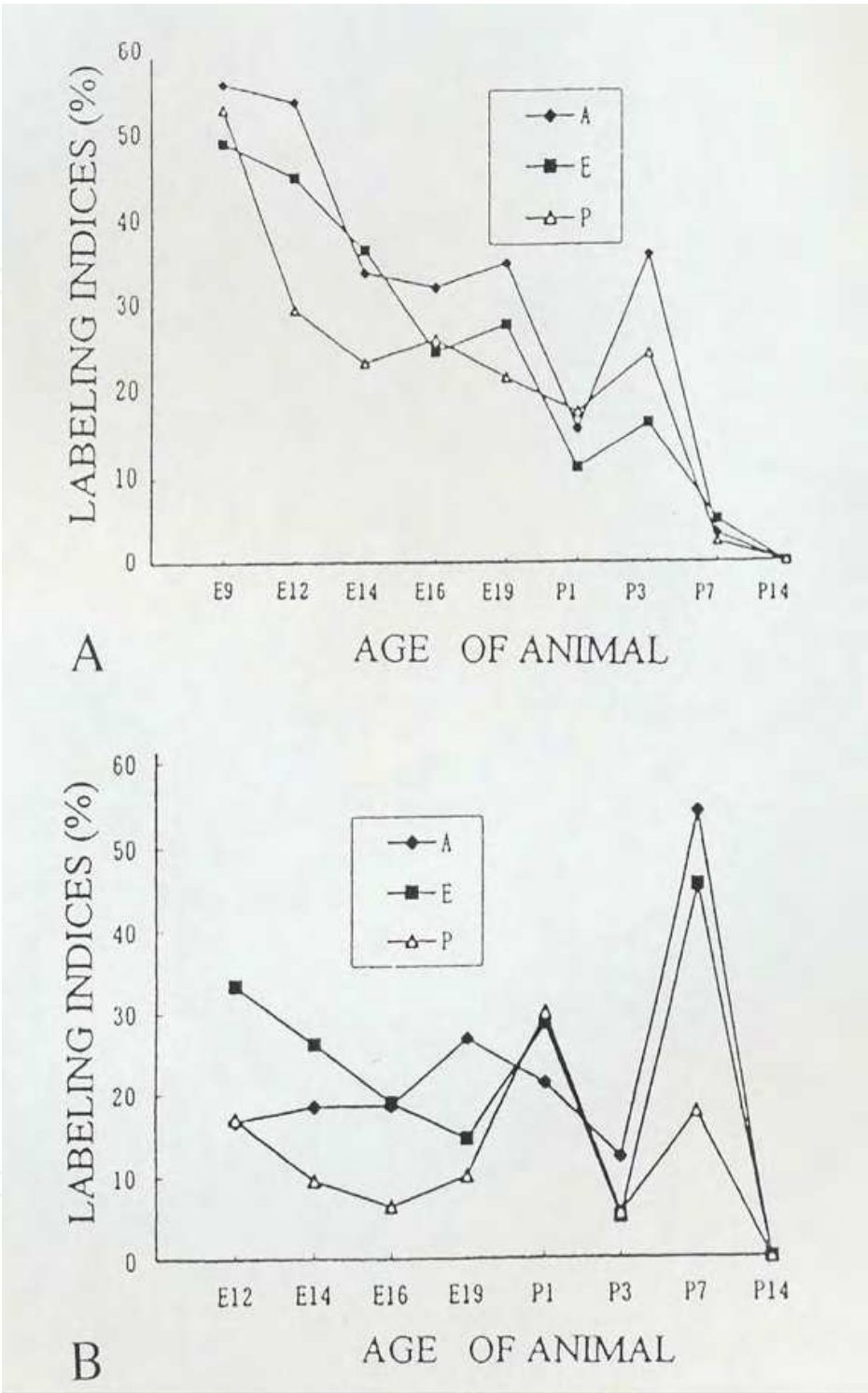


Fig. 29. Transitional curves of the labeling indices in the three regions (A: anterior, E: equator, P: posterior) of the retina and the pigment epithelium of aging mice labeled with ³H-thymidine, showing DNA synthesis. Mean ± Standard Deviation. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 191, 2002, Urban & Fischer, Jena, Germany

Fig. 29A. The labeling index in the retina.

Fig. 29B. The labeling index in the pigment epithelium.

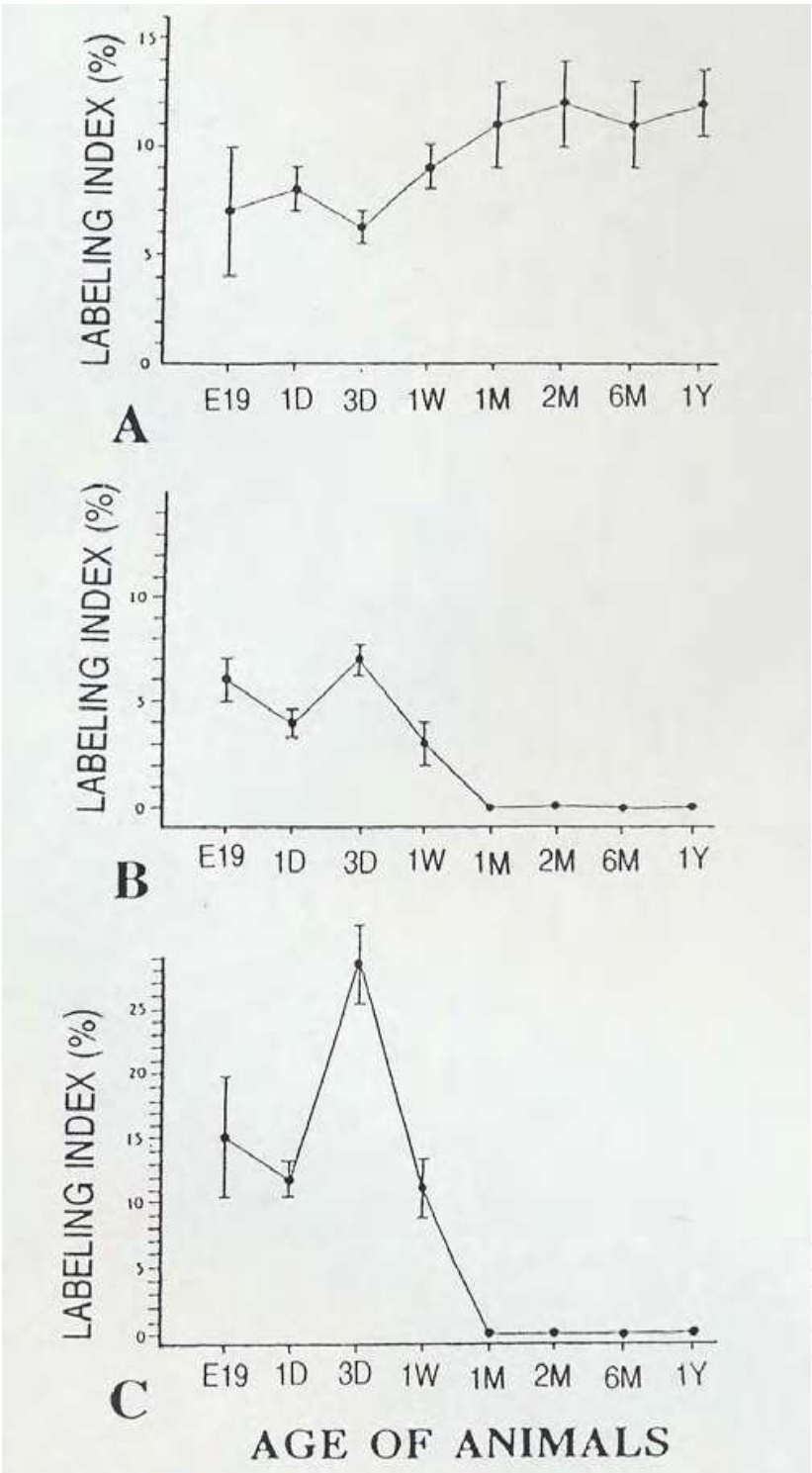


Fig. 30. Transitional curves of the labeling indices in the three layers of the central area of the cornea of aging mice labeled with ^3H -thymidine, showing DNA synthesis. Mean \pm Standard Deviation. From Nagata, T.: Radioautography, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p. 192, 2002, Urban & Fischer, Jena, Germany

Fig. 30A. The labeling index in the epithelium.

Fig. 30B. The labeling index in the stroma.

Fig. 30C. The labeling index in the endothelium.

In the corneas of aging mice, DNA synthesis was observed in all 3 layers, i.e., the epithelial, stromal and endothelial layers, at perinatal stages (Gao et al. 1993). The labeled cells with ^3H -thymidine were localized in the epithelial cells at prenatal day 19, postnatal day 1, 14 (Fig. 27G) to 1 year, while the labeled cells in the stromal and endothelial layers were less. The labeling index of the corneal epithelial cells reached a peak at 1 month after birth and decreased to 1 year, (Fig. 30A), while the indices of the stromal (Fig. 30B) and endothelial (Fig. 30C) cells were low and reached a peak at 3 days after birth and disappeared completely from postnatal 1 month to 1 year (Nagata 1999c).

In the ciliary body, the labeled cells were located in the ciliary and pigment epithelial cells, stromal cells and smooth muscle cells from prenatal day 19 to postnatal 1 week, but no labeled cells were observed in any cell types from postnatal day 14 to 1 year (Nagata et al. 1994). The labeling indices of all the cell types in the ciliary body were at the maximum at prenatal day 19 and decreased gradually after birth reaching 0 at postnatal day 14. On the other hand, when the ocular tissues were labeled with ^3H -uridine, silver grains appeared over all cell types at all stages of development and aging (Toriyama 1995, Nagata 2000f). The grain counts in the retina and the pigment epithelium increased from prenatal day 9 to postnatal day 1 in the retinal cells, while they increased from prenatal day 12 to postnatal day 7 in the pigment epithelial cells (Nagata 1999a,b, Nagata et al. 1994).

4.2 The DNA synthesis in the skin

The skin which covers the surface of the animal body can histologically be divided into 3 layers, the epidermis, the dermis and the hypodermis. We studied only the epidermal cells of young salamanders after hatching to 24 months during the aging by radioautography (Nagata 1998c). The fore-limbs and hind-limbs of salamanders were composed of skeletons consisting of bones and cartilages which were covered with skeletal muscles, connective tissues and epidermis consisting of stratified squamous epithelial cells in the outermost layer. We observed both the cartilage cells in the bone and the epithelial cells in the epidermis to compare the two cell populations. The skin of a salamander consisted of epidermis and dermis or corium which was lined with connective tissue layers designated as the subcutaneous layer. The former consisted of stratified squamous epithelium, while the latter consisted of dense connective tissues. The epithelial cells in the juvenile animals at 4 weeks after hatching were cuboidal in shape and not keratinized. Radioautograms labeled with ^3H -thymidine at this stage showed that many cells were labeled demonstrating DNA synthesis at both the superficial and deeper layers (Fig. 27H), resulting very high labeling index. At 6 weeks after hatching, the superficial cells changed their shape from cuboidal to flattened squamous, while the deeper and basal cells remained cuboidal. The numbers of labeled cells were almost the same as the previous stage at 4 weeks, but they were localized at the basal layer. The shape of epithelial cells in juvenile animals at 8, 9, 10, and 11 weeks differentiated gradually forming the superficial corneum layer which appeared keratinized and the deeper basal layer. Radioautograms at these stages showed that the labeled cells remarkably reduced as compared with that of 4 and 6 weeks. In the adult salamanders at 8 months up to 12 months, the dermal and epidermal cells showed complete mature structure and examination of radioautograms revealed that the labeled cells were localized at only the basal cell layer and their number reached very low but at constant level. No difference was found on the morphology and labeling between the fore-limbs and hind-limbs at any stages. Comparing the labeling indices of both epidermal cells and the cartilage cells in the limbs,

the labeling index of the epidermal cells was higher than the cartilage cells. The index of the dermal cells in the hind-limbs was at its maximum about 25% at 4 weeks, and fell down markedly with time from 6 weeks to 9 weeks. The labeling index of epidermal cells of the hind-limbs, on the other hand, had its maximum about 23% at 6 weeks, increasing from 20% at 4 weeks, and decreasing to about 18% at 8 weeks, then fell progressively with time, dropped to 5% at 9 weeks (Fig. 31). The labeling indices of the epidermal cells of both fore-limbs and hind-limbs were almost the same from 9 weeks to 12 months, keeping low constant level about 4-5%, but never reaching 0. These results indicated that the cutaneous cells belonged to the renewing cell population (Nagata 1998c).

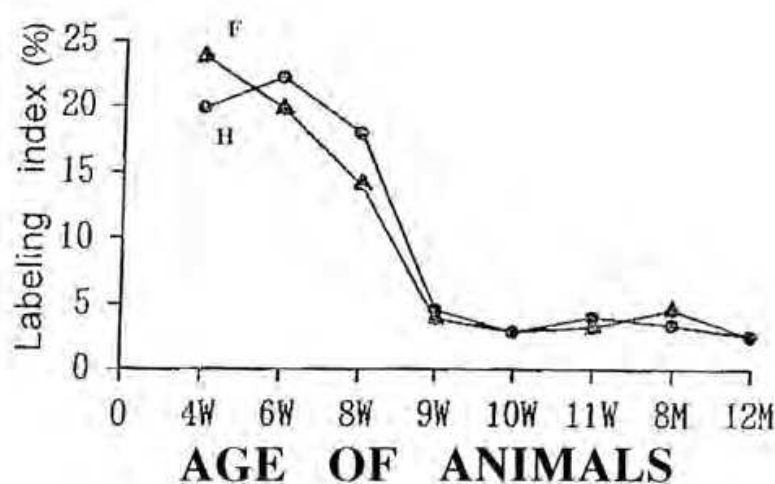


Fig. 31. Transitional curves of the labeling indices of the epithelial cells in the epidermis of the fore-limbs (F) and the hind-limbs (H) of the salamanders at various ages from 4 weeks to 12 months after hatching labeled with ^3H -thymidine. From Nagata, T.: Radioautographology, General and Special. In, Prog. Histochem. Cytochem. Vol. 37, No. 2, p.197, 2002, Urban & Fischer, Jena, Germany

4.3 The RNA synthesis in the sensory organs

We studied only the RNA synthesis in the chicken and mouse eyes among of the sensory organs.

4.3.1 The RNA synthesis in the eye

The RNA synthesis in the chicken eyes was studied with the ocular tissues of chicken embryos in incubation (Fig. 27D). Silver grains due to the incorporations of ^3H -uridine were observed over all the nuclei, cell organelles, cytoplasm of all the cells in the optic cups in development showing the RNA synthesis (Gunarso 1984a,b, Gunarso et al. 1969). Grain counting revealed that the counts gradually increased from day 2 to 7 and the numbers of silver grains were the most in the nuclei, while the numbers between the 3 portions of the optic cups, the anterior, equator and the posterior portions decreased from the anterior to the posterior at the same developmental stages (Gunarso 1984a).

On the other hand, the ocular tissues of aging mice were also labeled with ^3H -uridine. The silver grains demonstrating RNA synthesis appeared over all the cell types at all the stages of development and aging. The grain counts in the retina and the pigment epithelium increased from prenatal day 9 to postnatal day 1 in the retinal cells, while they increased from prenatal day 12 to postnatal day 7 in the pigment epithelial cells (Kong et al. 1992b).

On the other hand, the distribution and localization of TGF- β 1 and β FGF and their mRNA in the ocular tissues of aging mice were also studied (Nagata and Kong 1998). The posterior segment of BALB/c mouse eyes from embryonic day 14, 16, 19 and postnatal 1, 3, 5, 7, 14, 28, 42 and 70 were used. For in situ hybridization, ^{35}S -labeled oligonucleotide probes for TGF- β 1 and β FGF were used to detect their mRNA. Cryo-sections were picked up on glass slides which were processed for in situ hybridization and for radioautography. As the results, silver grains mainly located in the scleral layers and some in the choroidal and pigment epithelial layers, but only background level of grains were found in the whole retina. In the radioautograms from embryonic day 14 to adult mice at week 10 (day 70), the significant distribution of silver grains representing TGF- β 1 mRNA was not detected in the whole retina. However, the significant silver grains were detected in scleral and choroidal layers and mesenchymal cells at embryonic day 14, then the number of grains increased in these layers particularly in sclera from prenatal to postnatal neonate until adult (Fig. 27E). These results suggest that mRNA for TGF- β 1 and β FGF were synthesized in scleral, choroidal and pigment epithelial layers, but their proteins were transferred to the target cells of the retina and elsewhere. Furthermore, it is suggested that TGF- β 1 and β FGF may play important roles on retinal differentiation, development and aging, particularly during the late embryonic and newborn stages (Nagata and Kong 1998).

These results showed that RNA synthetic activities in the ocular cells changed due to the aging of individual animals.

4.4 The protein synthesis in the sensory organs

We studied only the protein synthesis in the mouse eyes among of the sensory organs.

4.4.1 The protein synthesis of the eye

The protein synthesis in the ocular tissues of aging mouse were studied in all the 3 layers of the eye, the tunica fibrosa, the tunica vasculosa and the tunica intima, or the cornea, ciliary bodies and the retina of the aging mouse at various stages after the administration of several precursors (Toriyama 1995, Nagata 1997c, 1999b,c,d, 2000e,f, 2001c, Nagata and Kong 1998, Cui et al. 2000).

The protein synthesis of the retina in aging mouse as revealed by ^3H -leucine incorporation demonstrated that number of silver grains in bipolar cells and photoreceptor cells was the most intense at embryonic stage and early postnatal days. The peak was 1 day after birth and decreased from 14 days to 1 year after birth. (Toriyama 1995). The protein synthesis of the cornea as revealed by ^3H -leucine incorporation (Nagata 1997c, 1999d, 2000f, 2001c, Nagata and Kong 1998, Cui et al. 2000) and the glycoprotein synthesis demonstrated by ^3H -glucosamine (Nagata et al. 1995) were also studied in several groups of aging ddY mice. Silver grains of both ^3H -leucine and ^3H -glucosamine incorporations were located in the epithelial cells, the stromal fibroblasts and the endothelial cells from prenatal day 19 to

postnatal 6 months. No silver grains were observed in the lamina limitans anterior (Bowman's membrane) and the lamina limitans posterior (Descemet's membrane). The grain densities by ^3H -leucine incorporation in 3 layers, i. e., epithelial, stromal and endothelial layers, increased from embryonic stage to postnatal day 3 and 7, then decreased to 2 weeks and 1 year. The grain densities due to the glycoprotein synthesis with ^3H -glucosamine were more observed in the endothelial cells of prenatal day 19 animals, but more in the epithelial cells of postnatal day 1, 3 and 7 animals. From the results, it was shown that the glycoprotein synthetic activity in respective cell types in the cornea of mouse changed with aging of the animals.

The collagen synthesis in the ocular tissues was also demonstrated by the incorporation of ^3H -proline in 4 groups of mice at various ages, from prenatal day 20, postnatal day 3, 7 and 30. The results showed that the sites of ^3H -proline incorporation were located in the stromal fibroblasts in both cornea and the trabecular meshworks in the iridocorneal angle in prenatal and postnatal newborn mice. No silver grains were observed in the epithelial and endothelial cells. On EM RAG, silver grains were localized over the endoplasmic reticulum and Golgi apparatus of fibroblasts and over intercellular matrices consisting of collagen fibrils. From the quantitative analysis, the grain densities were more observed in the fibroblasts in postnatal day 7 animals than younger animals at fetal day 20 and postnatal day 3, 7 and 30. In the same aging groups, the grain densities were more in the cornea than the iridocorneal angle. It was concluded that the collagen synthetic activity was localized in the fibroblasts in the cornea and the trabecular meshworks in the iridocorneal angle and the activity changed with aging, reaching the maximum at postnatal day 7.

On the other hand, the distributions of some of the ophthalmological drugs used for the treatment of human glaucoma patients were examined in the ocular tissues by LM and EM RAG (Nagata 2000f). However, its relationship to the aging was not studied.

4.5 The glucide synthesis in the sensory organs

We studied the aging changes of glucide synthesis by ^3H -glucosamine uptake in the ocular tissues of aging mice.

4.5.1 The glucide synthesis in the eye

The glycoprotein synthesis of the cornea in aging mouse as revealed by ^3H -glucosamine incorporation was studied in several groups of aging mice at various ages from prenatal stages to senescence (Nagata et al. 1995). Silver grains were located in the epithelial cells, the stromal fibroblasts and the endothelial cells from prenatal day 19 to postnatal 6 months. No silver grains were observed in the lamina limitans anterior (Bowman's membrane) and the lamina limitans posterior (Descemet's membrane). On the other hand, the grain densities by ^3H -leucine incorporation in 3 layers, i.e., epithelial, stromal and endothelial layers, increased from embryonic stage to postnatal day 3 and 7, then decreased to week 2 and year 1. The grain densities due to the glycoprotein synthesis with ^3H -glucosamine were more observed in the endothelial cells of prenatal day 19 animals, but more in the epithelial cells of postnatal day 1, 3 and 7 animals. From the results, it was shown that the glycoprotein synthetic activity in respective cell types in the cornea of mouse changed with aging of the animals.

5. Macromolecular synthesis in the tumor cells

We carried out several experiments dealing with the nucleic acid synthesis in some malignant tumor cells by means of LM and EM RAG.

5.1 The DNA synthesis in the tumor cells

The DNA synthesis in nuclei and mitochondria of cultured HeLa cells, an established cell line obtained from the carcinoma of the human uterus, or IgG myeloma cells from a human patient, labeled with ^3H -thymidine were demonstrated (Nagata 1972b,c,d, Fujii et al. 1980). The incorporations of these precursors increased or decreased depending upon the aging of isolated cells in vitro, i.e., the days of incubation in vitro. The incorporations of ^3H -thymidine demonstrating DNA synthesis increased immediately after the incubation and then gradually decreased due to incubation days, reaching zero within several days.

5.2 The RNA synthesis in the tumor cells

The RNA synthesis in nuclei and mitochondria of cultured HeLa cells, or IgG myeloma cells from a human patient, labeled with ^3H -uridine were demonstrated (Nagata 1972b,c,d, Fujii et al. 1980). The incorporations of these precursors increased or decreased depending upon the aging of isolated cells in vitro. The incorporations of ^3H -uridine demonstrating RNA synthesis increased immediately after the incubation and then gradually decreased due to incubation days.

6. Conclusions

From the results obtained, it was concluded that almost all the cells in various organs of all the organ systems of experimental animals at various ages from prenatal to postnatal development and senescence during the aging of cells and individual animals demonstrated to incorporate various macromolecular precursors such as ^3H -thymidine, ^3H -uridine, ^3H -leucine, ^3H -glucose or glucosamine, ^3H -glycerol and others localizing in the nuclei, cytoplasmic cell organelles showing silver grains due to DNA, RNA, proteins, glucides, lipids and others those which the cells synthesized during the cell aging. Quantitative analysis carried out on the numbers of silver grains in respective cell organelles demonstrated quantitative changes, increases and decreases, of these macromolecular synthesis in connection to cell aging of respective organs. In general, DNA synthesis with ^3H -thymidine incorporations in most organs showed maxima at perinatal stages and gradually decreased due to aging. To the contrary, the other syntheses such as RNA, proteins, glucides and lipids increased due to aging and did not remarkably decrease until senescence. Anyway, these results indicated that macromolecular synthetic activities of respective compounds in various cells were affected from the aging of the individual animals.

Thus, the results obtained from the various cells of various organs should form a part of special radioautographology that I had formerly proposed (Nagata 1999e, 2002), i.e., application of radioautography to the aging of cells, as well as a part of special cytochemistry (Nagata 2001), as was formerly reviewed. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future.

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