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Light and Electron Microscopic Radioautographic Studies on the Cell Aging of the Neuro-Sensory System of Mice

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Abstract

The term "Cell Aging" initially means how the cells change due to their aging. However, there are 2 meanings in this term, i.e., how a cell changes when it is isolated from original animals or plants such as *in vitro* cells in cell culture, otherwise how all the cells of an animal or a plant change *in vivo* due to the aging of the individual animal or plant.

We first studied the latter changes from the viewpoint of the cell nutrients, the precursors for the macromolecular synthesis such as DNA, RNA, proteins, glucides and lipids, which are incorporated and synthesized into various cells of individual animals. Therefore, this article deals with only the cell aging of animal cells *in vivo*, how the metabolism, i.e., incorporations and syntheses of respective nutrient precursors in various kinds of cells change due to the aging of individual experimental animals by means of microscopic radioautography to localize the RI-labeled precursors. The incorporations and syntheses of various precursors for macromolecules such as DNA, RNA, proteins, glucides, lipids and others in various kinds of cells of various organ. This review deals with the results of neuro-sensory system of mice only, i.e., the nervous system such as the brain, the spinal cord and the peripheral nerves as well as the sensory organs such as the eyes and the skin referring many original papers already published from our laboratory during these 60 years since the late 20C to early 21C.

Keywords: Cell aging; DNA; RNA; Proteins; Glucides; Lipids

Introduction

The term "Cell Aging" contains two meanings, one how a cell changes when it is isolated from *in vivo* original animals such as *in vitro* cells in cell culture, while the other means how all the cells of an animal change *in vivo* due to the aging of the individual animal. I had first studied the meaning of cell aging many years ago (more than 50 years) how a cell changed when it was isolated from original experimental animals such as mice and rats by cell culture [1-3], and then moved to the study on the latter cell aging, i.e., how all the cells of an experimental animal change *in vivo* due to the aging of the individual prenatal and postnatal animal [4-8].

Recently, we have been studying the aging changes from the viewpoint of the cell nutrients which were incorporated and synthesized into various cells in individual animals during their aging [9]. Therefore, this article deals with only the cell aging of animal cells *in vivo*, how the metabolism, i.e., incorporations and syntheses of respective nutrients, the macromolecular precursors, in various kinds of cells change due to the aging of individual experimental animals such as mice by means of microscopic radioautography. Among the incorporations and syntheses of various nutrients such as DNA, RNA, proteins, glucides, lipids and others in various kinds of cells of various organ in respective organ systems such as skeletal, muscular, circulatory, digestive, respiratory, urinary, reproductive, endocrine, nervous and sensory systems, this paper should review only the neuro-sensory system focusing on the nervous system and sensory system referring many original papers already published from our laboratory since 20C.

Radioautography

Since more than 50 years, we employed the specific techniques developed in our laboratory [10] that is designated as radioautography. The techniques employ both the physical techniques using RI-labeled compounds and the histochemical techniques treating tissue sections by coating sections containing RI-labeled precursors with photographic emulsions and processing for exposure and development. Such techniques can demonstrate both the soluble compounds diffusible in the cells and tissues and the insoluble compounds bound to the macromolecules [11].

In various kinds of cells of various organ in respective organ systems such as skeletal, muscular, circulatory, digestive, respiratory, urinary, reproductive, endocrine, nervous and sensory systems of the human and various animals, in order to observe the localizations of the incorporations and syntheses of various nutrients synthesizing macromolecules in the human or animal bodies such as DNA, RNA, proteins, glucides and lipids using RI-labeled compounds, to demonstrate the localizations of macromolecular synthesis by using such RI-labeled precursors as ^{3}H-thymidine for DNA, ^{3}H-uridine for RNA, ^{3}H-leucine for protein, ^{3}H-glucosamine or $^{35}SO_4$ for glucides and ^{3}H-glycerol for lipids are divided into macroscopic radioautography and microscopic radioautography.

As the results, specimens prepared for EM RAG are very thick and should be observed with high voltage electron microscopes in order to obtain better transmittance and resolution [12,13]. Such radioautographic techniques in details should be referred to other

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literature [10,14]. On the other hand, the systematic results obtained by radioautography should be designated as radioauographology, or science of radioautography [14-16]. This article deals with the results dealing with the radioautographic changes of individual cell by aging that should be included in radioautographology.

Macromolecular synthesis

The animal bodies, including both human body as well as the bodies of any experimental animals such as mice and rats consist of various macromolecules. They are classified into nucleic acids (both DNA and RNA), proteins, glucides and lipids, according to their chemical structures. These macromolecules can be demonstrated by specific histochemical staining techniques for respective molecules such as Feulgen reaction (Feulgen and Rossenbeck 1924) [17] that stains the entire DNA contained in the cells. Each compounds of macromolecules such as DNA, RNA, proteins, glucides, lipids can be demonstrated by respective specific histochemical staining [18] and such reactions can be quantified by microscpectrophotometry using specific wave-lengths demonstrating the total amount of respective compounds [19]. To the contrary, radioautography can only demonstrate the newly synthesized macromolecules such as synthetic DNA or RNA or proteins depending upon the RI-labeled precursors incorporated specifically into these macromolecules such as ^{3}H-thymidine into DNA or ^{3}H-uridine into RNA or ^{3}H-amino acid into proteins [10].

Therefore, the results of recent studies in our laboratory by the present author and co-workers concerning to the newly synthesized macromolecules should be reviewed in this article according to the histochemical classification of macromolecules as follows.

The DNA Synthesis

The DNA (deoxyribonucleic acid) contained in cells can be demonstrated either by biochemical techniques homogenizing tissues and cells or by morphological histochemical techniques staining tissue sections such as Feulgen reaction. To the contrary, the synthetic DNA or newly synthesized DNA but not all the DNA can be detected as macromolecular synthesis together with other macromolecules such as RNA or proteins in various organs of experimental animals by either morphological or biochemical procedures employing RI-labeled precursors. We have studied the sites of macromolecular synthesis in almost all the organs of mice during their aging from prenatal to postnatal development to senescence by means of microscopic radioautography, one of the morphological methods [20-28]. The results should be here described according to the order of organ systems in anatomy or histology.

The DNA 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 [29-35].

The DNA synthesis in the brains: The brains of mammals consist of the cerebrum, the cerebellum and the brain stem. We studied DNA synthesis and protein synthesis in the cerebellum of aging mice as well as the glucose incorporation in the cerebrum of adult gerbils [33,34]. 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 ^{3}H-thymidine, a DNA precursor, by LM and EM RAG [34]. The labeled nuclei, by the precursor, in both the neurons and glias, i.e., neuroblasts and glioblasts, were observed in the external granular layers of the cerebella of perinatal mice from embryonic day 19 (Figure 1A) to postnatal day 1, 3,

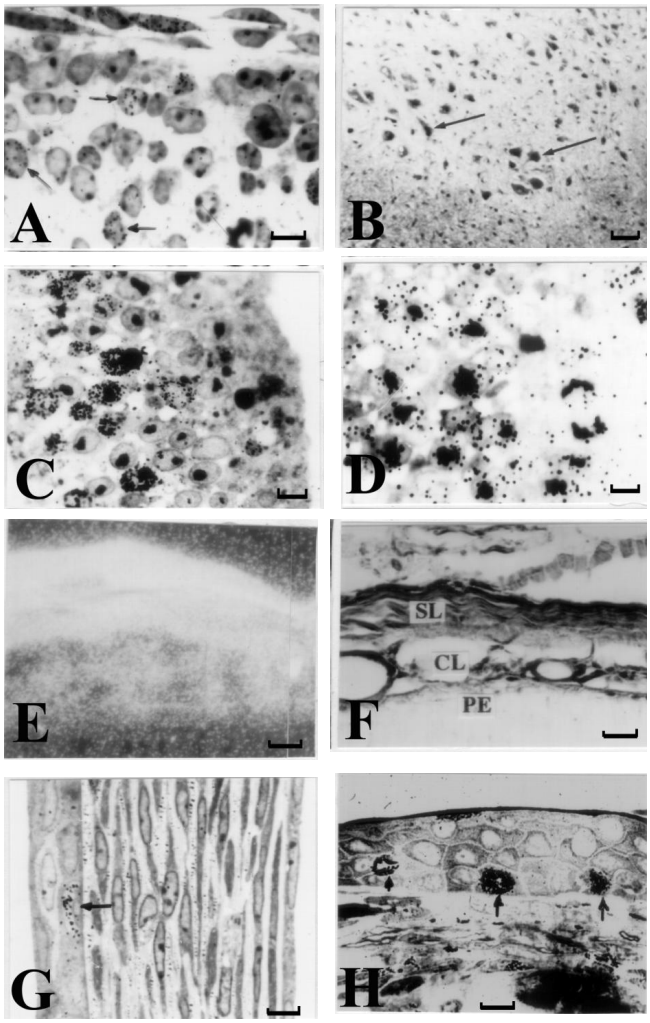

Figure 1: LM RAG of the neuro-sensory cells [64]

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

B: LM RAG of the spinal cord of a postnatal day 14 mouse immunostained with rabbit anti-TGF-b1 polyclonal IgG followed by ABC method, showing the ventral horn motoneurons are strongly positive. x70

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

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

E: Dark-field LM photo of the skleral 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-b1 mRNA. x450

F: Bright-field LM photo of the skleral 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-b1 mRNA. x450

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

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

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 (Figure 2A and 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.

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 adult 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 [29-32]. 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 ^{3}H-thymidine or ^{3}H-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 [29,30]. 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 gaglion 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.

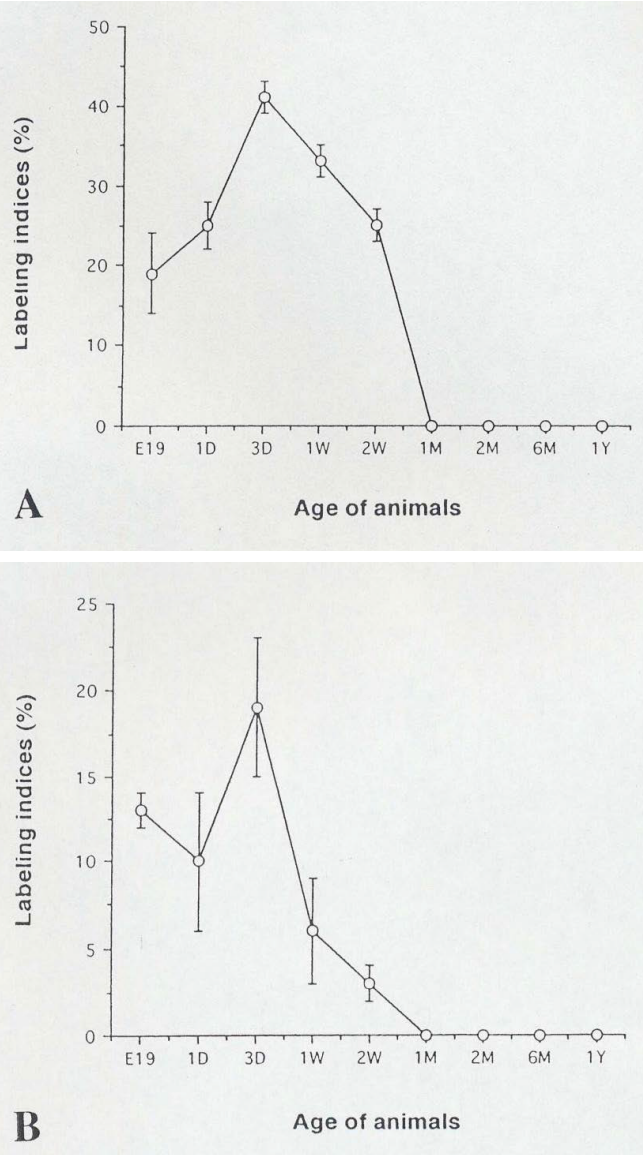

Figure 2: Transitional curves of the labeling indices of respective cell types in the cerebella of aging mice labeled with ^{3}H-thymidine, showing DNA synthesis. Mean ± Standard Deviation. [10]

A: The neuroblasts in the extragranular layer of the cerebella

B: The glioblasts in the extragranular layer of the cerebella

The DNA synthesis in the sensory system

The sensory system consists of five organs, i.e., the visual organ or the eye, stato-acoustic organ or the ear, gustatory organ or the tongue, olfactory organ or the nose, and the dermis or the skin. Among these sensory organs, we mainly studied the visual organ and the skin [14-16,36-59].

The DNA synthesis in the eye: The visual organ consists of the eye and its accessory organs. The eye of mammals consists of the cornea, iris, cilliary body, lens, retina, choroid and sclera. We studied mainly the macromolecular synthesis in the retina of chickens and mice [59]. 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 incubation by LM and EMRAG [36-45,48]. It was shown that the labelled cells with silver grains due to ^{3}H-thymidine were most frequently observed in the nuclei of the retinal cells in the posterior region of the day 2 chick embryo optic vesicle (Figure 1C) and the labelled cells moved from anterior to posterior regions. The number of labeled cells as expressed by labelling index (%), was more in the posterior regions than in the anterior and the equatorial regions and more in the outer portions than in the inner portions at day 2, but the labelling 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 (Figure 2). On the other hand, the silver

grains due to ^{3}H-uridine were observed over the nuclei and cytoplasm of all retinal cells from day 2 to 7 (Figure 1D) and the number of silver grains incorporating ^{3}H-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 [47]. On the other hand, DNA and RNA syntheses in the ocular tissues of aging ddY mice were also studied by Kong et al., Gao et al., Kong and Nagata [38,39,42,44]. 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 ^{3}H-thymidine *in vitro* and radioautographed [38-43,46]. 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 (Figure 3). The labeling indices of the retina and pigment epithelium were higher in earlier stages than in later stages, during which they steadily declined (Figure 3AB). 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 (Figure 3A). In the pigment epithelium (Figure 2B), 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 [38-41,43,44,51]. 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.

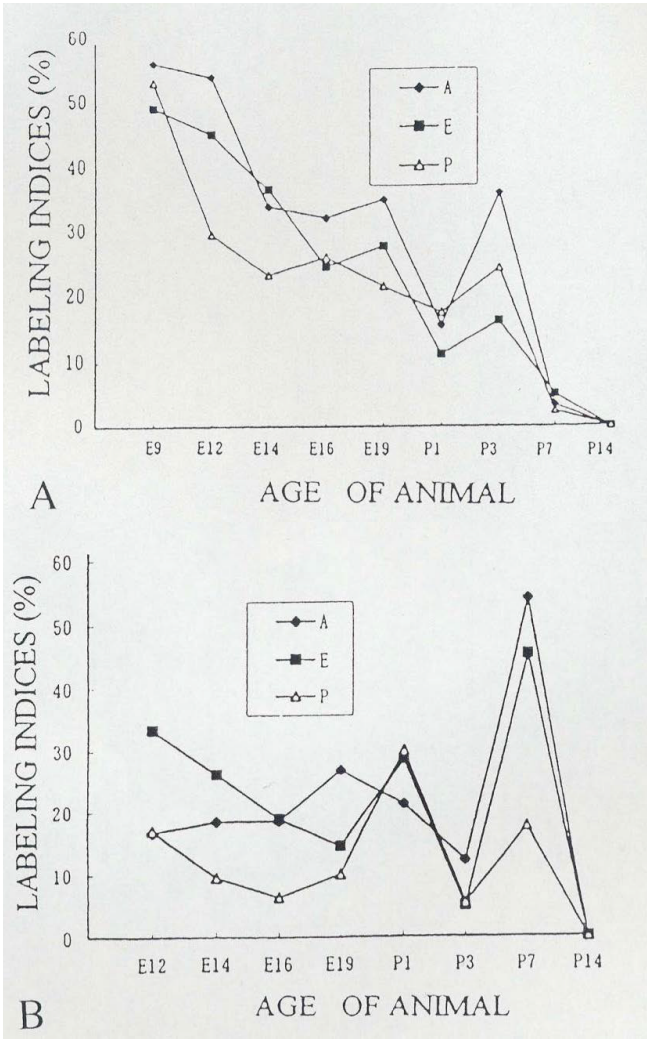

Figure 3: Transitional curves of the labeling indices in the three regions (A: anterior, E: eqauator, P: posterior) of the retina and the pigment epithelium aging mice labeled with ^{3}H-thymidine, showing DNA synthesis. Mean ± Standard Deviation. [10]

A: The labeling index in the retina

B: The labeloing index in the pigment epithelium

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 [42]. The labeled cells with ^{3}H-thymidine were localized in the epithelial cells at prenatal day 19, postnatal day 1, 14 (Figure 1G) 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, while the indices of the stromal and endothelial cells were low and reached a peak at 3 days after birth and disappeared completely from postnatal 1 month to 1 year [53].

In the cilliary body, the labeled cells were located in the cilliary 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 [45]. The labeling indices of all the cell types in the cilliary 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 ^{3}H-uridine, silver grains appeared over all cell types at all stages of development and aging [46,59]. The grain counts in the retina (Figure 2A) and the pigment epithelium (Figure 1B) 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 (Figure 4A-C) [45,51,52].

The DNA synthesis in the skin: The skin which covers the surface of the animal body can be divided into 3 layers, the epidermis, the dermis and the hypodermis. We studied only the epidermal cells of young salamanders after hatching by radioautography [50]. 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 ^{3}H-thymidine at this stage showed that many cells were labeled demonstrating DNA synthesis at both the superficial and deeper layers (Figure 1H), 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

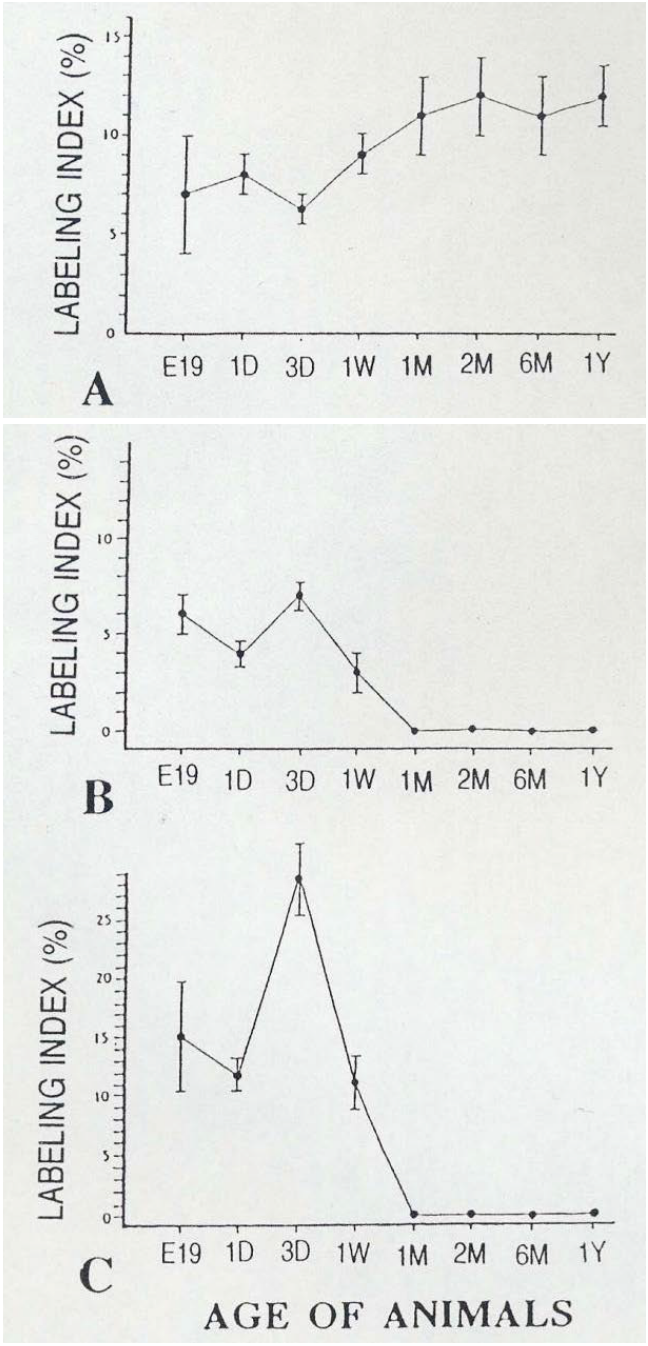

Figure 4: Transitional curves of the labeling indices in the three layers of the central area of the cornea of aging mice labeled with ^{3}H-thymidine, showing DNA synthesis. Mean ± Standard Deviation [10].

A: The labeling index in the epithelium

B: The labeling index in the stroma

C: The labeling index in the endothelium

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 labelled 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. 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 cell belonged to the renewing cell population (Nagata 1998c).

The RNA Synthesis

The synthetic RNA or newly synthesized RNA but not all the RNA in the cells can be detected as macromolecular synthesis together with other macromolecules such as DNA or proteins in various organs of experimental animals by either biochemical or morphological procedures employing RI-labelled precursors. To the contrary, the RNA (ribonucleic acid) contained in cells can be demonstrated either by biochemical techniques homogenizing tissues and cells or by morphological histochemical techniques staining tissue sections such as methyl green-pyronin staining. We have studied the sites of macromolecular synthesis in almost all the organs of mice during their aging from prenatal to postnatal development to senescence by means of microscopic radioautography, one of the morphological methods [20-22,24-26,28]. The results obtained from RNA synthesis should be here described according to the order of organ systems in anatomy or histology. In contrast to the results obtained from DNA synthesis of almost all the organs, we have studied only several parts of the organ systems. The skeletal system, the muscular system or the circulatory system were not so much studied yet.

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* hybridizaion.

The RNA synthesis in the spinal cord: The localization of TGF-b1 mRNA in the segments of the spinal cords of mice was investigated by means of in situ hybridization techniques together with immunohistochemical staining [51]. 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-b1 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-b1 mRNA was detectable in the meninges surrounding the spinal cord, but scarcely detected in spinal cord parenchyma (Figure 1B). The localization of TGF-b1 mRNA in the spinal cord suggested that TGF-b1 acted through paracrine mechanism in the morphogenesis of the spinal cord in mice. The localization of TGF-b1 and its mRNA in

the segments of the spinal cords of mice was also investigated with immunocytochemical techniques [51,52]. 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-b1 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 (Figure 1B) 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-b1 regulates motoneuron growth and differentiation as well as they was probably correlated with formation, differentiation and regeneration of myelin of nerve tracts. The immunostaining with bFGF antibody presented the same basal pattern as shown in TGFb1 immunocytochemistry [60]. The positive immunoreactivities were detected in ganglion cell layer, inner and outer plexiform layers, retinal pigment epithelial layer, choroidal and scleral layers. Since TGF-b1 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-b1 polypeptide and TGF-b1 mRNA in the spinal cord suggest that TGF-b1 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.

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

The RNA synthesis in the eye: The RNA synthesis in the chicken eyes was studied with the ocular tissues of chicken embryos in incubation (Figure 1D). Silver grains due to the incorporations of ^{3}H-uridine were observed over all the nuclei, cell organelles, cytoplasm of all the cells in the optic cups in development showing the RNA synthesis [36,37,47]. 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 [36].

On the other hand, the ocular tissues of aging mice were also labeled with ^{3}H-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 [39].

On the other hand, the distribution and localization of TGF-b1 and b FGF and their mRNA in the ocular tissues of aging mice were also studied [60]. 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-b1 and bFGF were used to detect their mRNA. Cryo-sections were picked up on glass slides that 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-b1 mRNA was not detected in the whole retina. However, the significant silver grains were detected in scleral and chorioidal 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 (Figure 1E). These results suggest that mRNA for TGF-b1 and bFGF 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-b1 and bFGF may play important roles on retinal differentiation, development and aging, particularly during the late embryonic and newborn stages [60].

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

The Protein Synthesis

The proteins found in animal cells are chemically classified into two, i.e., the simple proteins and the conjugated proteins. Therefore, the proteins can be demonstrated by showing specific reactions to respective amino-acids composing any proteins. They are hitochchemically composed of various amino-acids which initially form low molecular polypeptides and finally macromolecular compounds designated as proteins. Thus, the proteins contained in cells can be demonstrated either by morphological histochemical techniques staining tissue sections such as Millon reaction or tetrazonium reaction or otherwise by biochemical techniques homogenizing tissues and cells. To the contrary, the newly synthesized proteins but not all the proteins in the cells can be detected as macromolecular synthesis together with other macromolecules such as DNA or RNA in various organs of experimental animals by either morphological or biochemical procedures employing RI-labelled precursors. We have studied the sites of macromolecular synthesis in almost all the organs of mice during their aging from prenatal to postnatal development to senescence by means of microscopic radioautography [10,20-22,24,25,28]. The results obtained from protein synthesis should be described according to the order of organ systems in anatomy or histology. In contrast to the results obtained from DNA synthesis of almost all the organs, we have studied only several parts of the organ systems yet.

The Protein synthesis in the nervous system

We studied the protein synthesis in the nervous system of aging mice in the cerebella and the spinal cords of mice at various ages from prenatal to postnatal 2 years.

The protein synthesis in the cerebellum of aging mouse: 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 ^{3}H-leucine, protein syntheses of both neuroblasts and glioblasts were observed by LM and EM RAG in the extragranular layers of perinatal animals [34,61,62]. The silver grains due to ^{3}H-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, and 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.

The protein synthesis in the sensory system: Among the several

sensory organs, we studied only the ocular tissues of aging mice at various ages from perinatal to senescent stages.

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, cilliary bodies and the retina of the aging mouse at various stages after the administration of several precursors [14,46,52-54,59,60,61,63,64].

The protein synthesis of the retina in aging mouse as revealed by ^{3}H-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 [46]. The protein synthesis of the cornea as revealed by ^{3}H-leucine incorporation [54,59,60,63,64] and the glycoprotein synthesis demonstrated by ^{3}H-glucosamine [65] were also studied in several groups of aging ddY mice. Silver grains of both ^{3}H-leucine and ^{3}H-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 ^{3}H-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 ^{3}H-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 ^{3}H-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 [59]. However, its relationship to the aging was not studied.

The Glucide Synthesis

The glucides found in animal cells and tissues are histochemically composed of various low molecular sugars such as glucose or fructose called monosaccharides which form compounds of polysaccharides or complex mucopolysaccharides connecting to sulfated compounds. The former are called simple polysaccharides, while the latter mucopolysubstances. Thus, the glucides are chemically classified into 3 groups, monosaccharides such as glucose or fructose, disaccharides such as sucrose and polysaccharides such as mucosubstances. However, in most animal cells polysaccharides are much more found than monosaccharides or disaccharides. The polysaccharides can be classified into 2, i.e. the simple polyscaccharides and the mucosubstances. Anyway, they are composed of various low molecular sugars that can be demonstrated by either biochemical techniques or histochemical reactions. To the contrary, the newly synthesized glucides but not all the glucides in the cells and tissues can be detected as macromolecular synthesis together with other macromolecules such as DNA, RNA or proteins in various organs of experimental animals by either biochemical or morphological procedures employing RI-labelled precursors. We have studied the sites of macromolecular synthesis in almost all the organs of mice during their aging from prenatal to postnatal development to senescence by means of microscopic radioautography [10,20-22,24,28,66]. The results obtained from glucides synthesis are described according to the order of organ systems in anatomy or histology. In contrast to the results obtained from DNA synthesis of almost all the organs, we have studied only several parts of the organ systems. The skeletal system, the muscular system and the circulatory system were not yet studied.

The glucide synthesis in the nervous system

The incorporation of ^{3}H-deoxyglucose was studied in the adult gerbil brains among the nervous system of experimental animals [33]. The changes of soluble deoxyglucose uptake in the hippocampus were studied after ^{3}H-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 ^{3}H-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.

The glucide synthesis in the sensory system

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

The glucide synthesis in the eye: The glycoprotein synthesis of the cornea in aging mouse as revealed by ^{3}H-glucosamine incorporation was studied in several groups of aging mice at various ages from prenatal stages to senescence [65]. 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 ^{3}H-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 ^{3}H-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 Lipid Synthesis

The lipids found in animal cells are histochemically composed of various low molecular fatty acids. They are esters of high fatty acids and glycerol that can biochemically be classified into simple lipids and

compound lipids such as phospholipids, glycolipids or proteolipids. The simple lipids are composed of only fatty acids and glycerol, while the latter composed of lipids and other components such as phosphates, glucides or proteins. In order to demonstrate intracellular localization of total lipids, we can employ either biochemical techniques or histochemical reactions. To the contrary, the newly synthesized lipids but not all of the lipids in the cells can be detected as macromolecular synthesis similarly to the other macromolecules such as DNA, RNA, proteins or glucides in various organs of experimental animals by either morphological or biochemical procedures employing RI-labeled precursors. We have studied the sites of macromolecular synthesis in almost all the organs of mice during their aging from prenatal to postnatal development to senescence by means of microscopic radioautography [10,20-24,28,66,67,10]. However, we have not yet studied the lipids synthesis so much as compared to other compounds. We have studied only a few organs of the digestive system.

The Intracellular Localization of the other Substances

The other substances than macromolecules that can also be demonstrated by radioautography are target tracers not the precursors for the macromolecular synthesis. They are hormones such as ^{3}H-methyl prednisolone [68], neurotransmitters and inhibitors such as ^{14}C-bupranolol, a beta-blocking agent [69] or ^{3}H-befunolol [70,71], vitamins, drugs such as synthetic anti-allergic agent ^{3}H-tranilast [72-76], hypolipidemic agent bezafibrate [77-79], calmodulin antagonist [80,81] or anti-hypertensive agent ^{3}H-benidipine hydrochloride [82], toxins, inorganic substances such as mercury [83] and others such as laser beam irradiation [84]. The details are referred to the previous publication on the radioatuographology [10]. However, their relationships to the cell aging were not yet studied.

Conclusion

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 ^{3}H-thymidine, ^{3}H-uridine, ^{3}H-leucine, ^{3}H-glucose or glucosamine, ^{3}H-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 this 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 synthesis 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 [16], i.e., application of radioautography to the aging of cells, as well as a part of special cytochemistry [12], as was formerly reviewed. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future by other authors.

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