Original Article: Electron Microscopic Radioautographic Studies on the RNA Synthesis in the Colonic Epithelial Cells of Aging Mice

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Abstract: For the purpose of studying the aging changes of macromolecular synthesis in the colonic cells of experimental animals, we studied 10 groups of aging mice during aging from fetal day 19 to postnatal month 24. They were injected with ^{3}H-uridine, a precursor for RNA synthesis, sacrificed and the colonic tissues were taken out, fixed and processed for light and electron microscopic radioautography. On many radioautograms the localization of silver grains demonstrating RNA synthesis in colonic epithelial cells in respective aging groups were analyzed qualitatively. The number of mitochondria per cell, the number of labeled mitochondria with silver grains in each cell in respective aging groups were analyzed qualitatively and quantitatively in relation to the aging of animals.

The results revealed that the RNA synthesis as expressed by the number of silver grains in cell nuclei, cell organelles, changed with the aging of animals. It was demonstrated that the number of mitochondria increased from embryonic day 19 to postnatal newborn day 1, 3, 7, 14, adult month 1 and 2, reaching the maximum, then decreased to senile year 1 to 2. On the other hand, the number of labeled mitochondria showing RNA synthesis at various ages increased from embryonic day 19 to postnatal newborn day 1, 3, 7, 14, adult month 1 and 2, reaching the maximum and decreased slightly to senile month 6 to senile year 1 and 2. To the contrary, the labeling index increased from embryonic day 19 to postnatal day 1, 2 and 3, then decreased to day 14 and month 1, and increased again to month 6, 12 and month 24, indicating the aging changes. These results demonstrated that intramitochondrial RNA synthesis in the colonic epithelial cells increased, then decreased and again increased due to aging of individual animals depending upon the cellular activities at respective aging stages. Based upon our findings, available literatures on macromolecular synthesis in mitochondria of various cells are reviewed.

1. INTRODUCTION

The colon is a part of the large intestines in animals and men, among the digestive tubes between the small intestines and rectum. The mucous membrane of the colon does not form folds like the small intestines or the last portion of the large intestine, i.e. the rectum. The colonic mucous membrane consists of the simple columnar epithelium forming intestinal crypts but not such villi as in the small intestines. Therefore, the colonic mucous membrane has a smooth surface, that is lined by simple columnar epithelium with a thin striated border. The intestinal crypts, or the glands of Lieberkuehn are straight tubules, which attain a greater length in the colon than in the small intestines. The colonic epithelial cells differ from the small intestines in their greater abundance of goblet cells. The epithelial cells proliferate at the bottom of the crypts where undifferentiated proliferating cells exists.

We have studied the macromolecular synthesis of the colonic epithelial cells in the aging mice in 10 groups of litter mates of both sexes, each 3, from embryonic day 19 to postnatal day 1, 3, 7, 14, month 1, 2, 6, 12 (year 1) and 24 (year 2), by means of light and electron microscopic radioautography. We first studied the DNA synthesis in the colon and caecum of aging mice from embryonic day 19 to postnatal month 12 by using ^{3}H-thymidine [1-3]. Light and electron microscopic radioautograms (LM and EM RAG) of the colonic and caecal epithelia revealed that some of the nuclei of columnar epithelial cells were labeled with ^{3}H-thymidine showing DNA synthesis. The labeled cells were located at the lower half of the crypts, and the labeling index (LI) changed with the aging. A peak of the labeling index of the absorptive cells was found at embryonic day 19, but decreased at the postnatal day 1 and then kept an almost constant value until postnatal month 12. On the other hand, the LI of the goblet cells showed the peak at embryonic day 19, then decreased gradually with aging from postnatal day 1 and completely disappeared from postnatal month 1 onwards, while the basal granulated cells were located only in the base of crypts and the significant difference of LI was not found from embryonic day 19 to postnatal month 12. However, the localizations of silver grains over the mitochondria of these cells were not examined in these studies [1-3]. In the previous studies, we observed the DNA synthesis in the mitochondria of

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columnar epithelial cells, mainly the columnar absorptive cells, in 10 groups of litter mate mice, from embryonic day 19, postnatal day 1, 3, 7, 14, and month 1, 6, 12 (year 1) and 24 (2 years), where the number of labeled cells were counted and the labeling indices of these cells were analyzed.

On the other hand, in contrast to the DNA synthesis in nuclei in various cells of colonic and cecal epithelial cells in aging mice, we also found the silver grains due to DNA and RNA syntheses in mitochondria of various cells such as the pancreatic acinar cells, hepatic cells, adrenal cells or renal cells showing intramitochondrial DNA and RNA syntheses [4-7]. We later found that the activities of DNA and RNA syntheses in mitochondria of various cells changed due to aging of individual animals [8-11].

Thus, we have formerly concentrated to study the intramitochondrial DNA and RNA as well as protein synthesis in various cells of aging mice [12], especially in the livers which contained many mitochondria [13]. To the contrary, this paper deals with the intramitochondrial RNA synthesis in colonic epithelial cells of aging ddY mice at various ages in 10 groups during development and aging from prenatal embryo day 19 to postnatal 2 years at senescence.

2. MATERIALS AND METHODS

2.1. The Experimental Animals

The colonic tissues were obtained from 10 groups of aging normal ddY strain mice, each consisting of 3 litter mates of both sexes, total 30, from prenatal embryo day 19 to newborn postnatal day 1, 3, 7, 14, adult at month 1, 2, 6, 12 (year 1) to month 24 (year 2). All the animals were housed under conventional conditions and bred with normal diet (mouse chow Clea EC2, Clea Co., Tokyo, Japan) with access to water ad libitum in our laboratory. They were administered with ^{3}H-uridine, an RNA precursor, and the colonic tissues were taken out, fixed and processed for electron microscopic radioautography. All the procedures used in this study concerning the animal experiments were in accordance with the guidelines of the animal research committee of Shinshu University School of Medicine where this experiment was carried out as well as the principles of laboratory animal care in NIH publication No. 86-23 (revised 1985).

2.2. Procedures of Microscopic Radioautography

All the animals were injected intraperitoneally with ^{3}H-uridine (Amersham, England, specific activity 877 GBq/mM) in saline, at 9 a.m., one hour before sacrifices. The dosage of injections was 370 KBq/gm body weight. The animals were perfused at 10 a.m., one hour after the injection, *via* the left ventricles of the hearts with 0.1 M cacodylate-buffered 2.5% glutaraldehyde under Nembutal (Abbott Laboratories, Chicago, ILL, USA) anesthesia. The distal colon was taken out from each animal, excised into small tissue pieces of the colonic tissues (size 1mm x 1mm x 1mm) which were immersed in the same fixative at 4˚C for 1 hr., followed by postfixation in 1% osmium tetroxide in the same buffer at 4˚C for 1 hr., dehydrated in graded series of ethanol and acetone, and embedded in epoxy resin Epok 812 (Oken, Tokyo, Japan).

For light microscopic radioautography, semithin sections at 0.5μm thickness, thicker than conventional ultrathin sections in order to shorten the exposure time for radioautography, were cut in sequence on a Porter-Blum MT-2B ultramicrotome (Dupont-Sorvall, Newtown, MA, USA) using glass knives. The sections were collected on collodion coated glass slides, coated with Konica NR-M2 radioautographic emulsion (Konica, Tokyo, Japan) by a dipping method [5-7]. They were stored in dark boxes containing silica gel (desiccant) at 4˚C for exposure. After the exposure for 2 months, the specimens were processed for development in freshly prepared D-19 solution for 10 min at 16˚C in a water bath, rinsed in distilled water and dried in an oven at 37˚C overnight, stained with toluidine blue solution for 2 min and dried for light microscopy.

For electron microscopic radioautography, semithin sections at 0.2μm thickness, thicker than conventional ultrathin sections in order to shorten the exposure time for radioautography, were cut in sequence on a Porter-Blum MT-2B ultramicrotome (Dupont-Sorvall, Newtown, MA, USA) using glass knives. The sections were collected on collodion coated copper grid meshes (VECO, Eerbeek, Netherlands), coated with Konica NR-H2 radioautographic emulsion (Konica, Tokyo, Japan) by a wire-loop method [5-7]. They were stored in dark boxes containing silica gel (desiccant) at 4˚C for exposure. After the exposure for 10 months, the specimens were processed for development in freshly prepared gold latensification solution for 30 sec at 16˚C and then in fresh phenidon developer for 1 min at 16˚C in a water bath, rinsed in distilled water and dried in an oven at 37˚C overnight, stained with lead citrate solution for 3 min, coated with carbon for electron microscopy. The electron microscopic (EM) radioautograms were examined in a JEOL JEM-4000EX electron microscope (JEOL, Tokyo, Japan) at

accelerating voltages of 400 kV for observing thick specimens.

2.3. Quantitative Analysis of Light Micrographs

For quantitative analysis of light micrographs, twenty LM radioautograms showing cross sections of colonic columnar absorptive cells from each group, based on the light microscopic photographs taken after observation on at least 100 colonic epithelial cells from respective animals were analyzed to calculate the total number of labeled nuclei covered with silver grains by visual grain counting.

On the other hand, the number of silver grains in the same area size as a nucleus outside cells was also calculated in respective specimens as background fog, which resulted in less than 1 silver grain (0.02/nuclear area) almost zero. Therefore, the grain count in each specimen was not corrected with background fog. From all the data thus obtained the averages and standard deviations in respective aging groups were computed with a personal computer (Macintosh type 8100/100, Apple Computer, Tokyo, Japan). The data were stochastically analyzed using variance and Student's t-test. The differences were considered to be significant at P value <0.01.

2.4. Quantitative Analysis of Electron Micrographs

For quantitative analysis of electron micrographs, twenty EM radioautograms showing cross sections of colonic columnar absorptive cells from each group, based on the electron microscopic photographs taken after observation on at least 100 colonic epithelial cells from respective animals were analyzed to calculate the total number of mitochondria in each cell, and the number of labeled mitochondria covered with silver grains by visual grain counting.

On the other hand, the number of silver grains in the same area size as a mitochondrion outside cells was also calculated in respective specimens as background fog, which resulted in less than 1 silver grain (0.02/mitochondrial area) almost zero. Therefore, the grain count in each specimen was not corrected with background fog. From all the data thus obtained the averages and standard deviations in respective aging groups were computed with a personal computer (Macintosh type 8100/100, Apple Computer, Tokyo, Japan). The data were stochastically analyzed using variance and Student's t-test. The differences were considered to be significant at P value <0.01.

3. RESULTS

3.1. Morphological Observations

The colonic tissues obtained from ddY strain mice at various ages from embryo day 19 to postnatal month 24, consisted of 3 layers, i.e., the mucous layer, the muscular layer and the serous membrane as observed by both light and electron microscopy. The mucous layer of the colon does not form folds like the small intestines or the last portion of the large intestine, i.e. the rectum. The colonic mucous layer consists of the mucous membrane and the submucous tissues. The former consists of the simple columnar epithelium forming intestinal crypts but not such villi as in the small intestines, while the latter consists of the connective tissues. Therefore, the colonic mucous membrane has a smooth surface, that is lined by simple columnar epithelium with a thin striated border. The intestinal crypts, or the glands of Lieberkuehn are straight tubules, which attain a greater length in the colon than in the small intestines. The colonic epithelial cells differ from the small intestines in their greater abundance of goblet cells. The epithelial cells proliferate at the bottom of the crypts where undifferentiated proliferating cells exist.

We have studied the macromolecular synthesis of the colonic epithelial cells in the aging mice in 10 groups of litter mates of both sexes, each 3, from embryonic day 19 to postnatal day 1, 3, 7, 14, month 1, 2, 6, 12 (year 1) and 24 (year 2), by means of light and electron microscopic radioautography. We first studied the DNA synthesis in the colon and caecum of aging mice from embryonic day 19 to postnatal month 12 by using ^{3}H-thymidine [1-3]. Light and electron microscopic radioautograms (LM and EM RAG) of the colonic and caecal epithelia revealed that some of the nuclei of columnar epithelial cells were labeled with ^{3}H-thymidine showing DNA synthesis. The labeled cells were located at the lower half of the crypts, and the labeling index (LI) changed with the aging. A peak of the labeling index of the absorptive cells was found at embryonic day 19, but decreased at the postnatal day 1 and then kept an almost constant value until postnatal month 12. On the other hand, the LI of the goblet cells showed the peak at embryonic day 19, then decreased gradually with aging from postnatal day 1 and completely disappeared from postnatal month 1 onwards, while the basal granulated cells were located only in the base of crypts and the significant difference of LI was not found from embryonic day 19 to postnatal month 12. However, the localizations of silver grains

over the mitochondria of these cells were not examined in these studies.

In the present study, we observed the RNA synthesis in the mitochondria of columnar epithelial cells, mainly the columnar absorptive cells, in 10 groups of litter mate mice, from embryonic day 19, postnatal day 1, 3, 7, 14, and month 1, 6, 12 (year 1) and 24 (2 years) and the number of labeled cells were counted and the labeling indices of these cells were analyzed. Among the epithelial cells covering the colon, the columnar absorptive cells at the bottom of the crypts were analyzed in this study.

3.2. Radioautographic Observations

Observing electron microscopic radioautograms of the columnar epithelial cells, the silver grains were found over the nuclei as well as over the cytoplasm including mitochondria of some columnar epithelial cells (Figures **1-7**), labeled with ^{3}H-uridine, demonstrating RNA synthesis at respective aging stages from perinatal stages at embryonic day 19 (Figure **1**), to postnatal day 1 (Figure **2**) and day 3 and 7 (Figure **3**), and day 14, to adult stage at month 1 (Figure **4**), month 2 and 6 (Figure **5**), and to senescent stage at month 12 (Figure **6**) and 24 (Figure **7**).

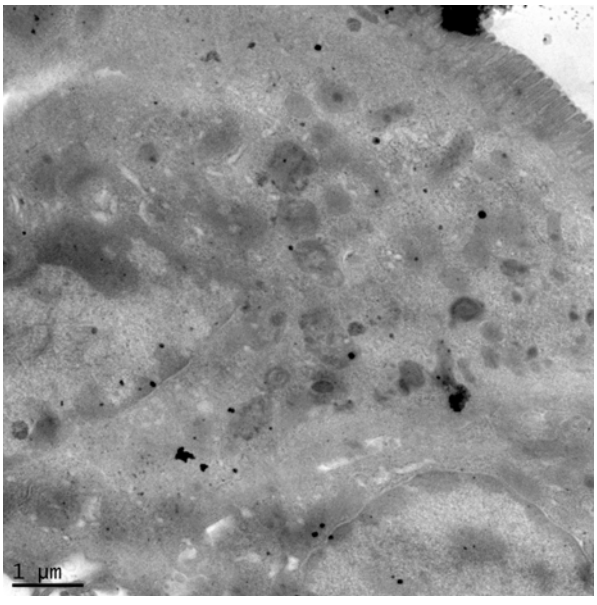


Figure 1: Electron microscopic radioautograms (EMRAG) of the undifferentiated columnar epithelial cells, labeled with ^{3}H-uridine, demonstrating RNA synthesis at embryonic day 19. The silver grains were found over the nuclei as well as over the cytoplasm including mitochondria of some columnar epithelial cells. x2,000.

The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices similarly to other cells such as in the livers [13] or the adrenal glands [14] as reported previously.

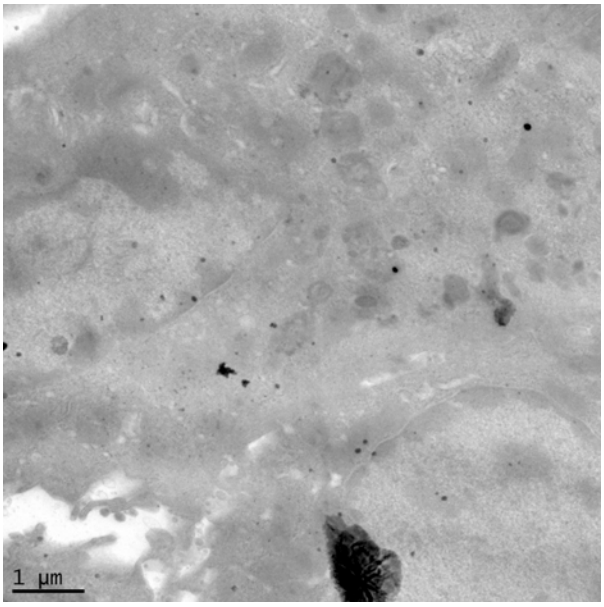


Figure 2: EM RAG of the differentiated columnar epithelial cells of a postnatal day 1 mouse labeled with ^{3}H-uridine, showing silver grains over the nuclei and mitochondria. x3,000.

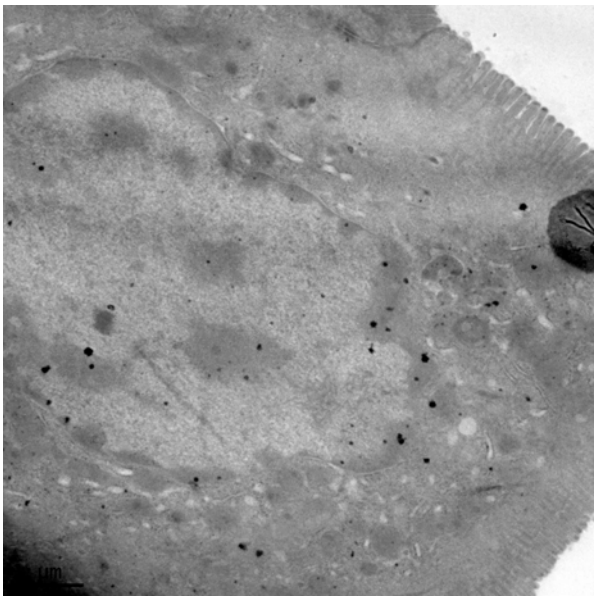


Figure 3: EM RAG of several columnar epithelial cells of a postnatal day 7 mouse labeled with ^{3}H-uridine, showing silver grains over the nuclei and mitochondria. x6,000.

3.3. Quantitative Analysis

3.3.1. Number of Mitochondria Per Cell

Preliminary quantitative analysis on the number of mitochondria in 10 columnar epithelial cells whose nuclei were labeled with silver grains and other 10 cells

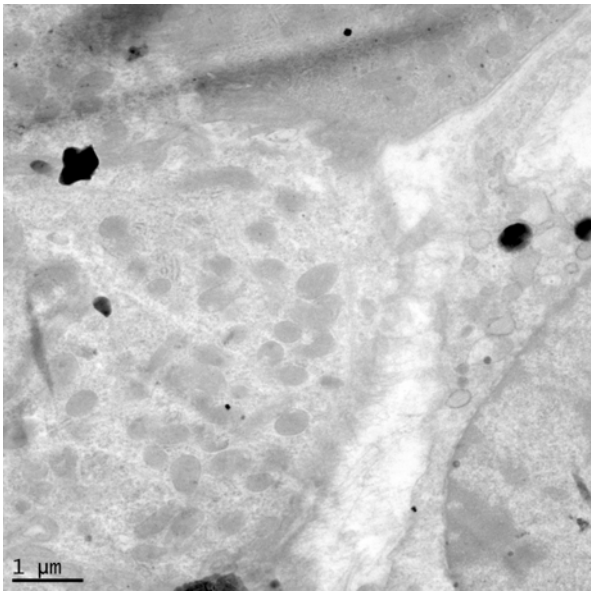


Figure 4: EM RAG of several columnar epithelial cells including a few goblet cells of a postnatal month 1 mouse labeled with ^{3}H-uridine, showing silver grains over the nuclei and mitochondria. x6,000.

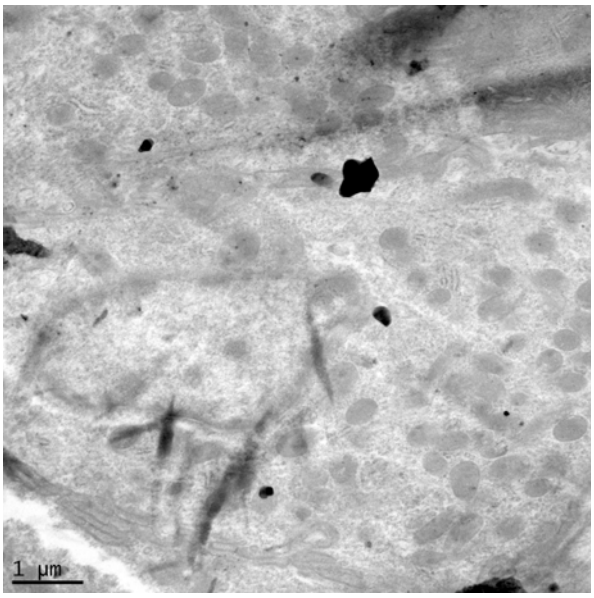


Figure 5: EM RAG of several columnar epithelial cells including a few goblet cells of a postnatal month 6 mouse labeled with ^{3}H-uridine, showing silver grains over the nuclei and mitochondria. x6,000.

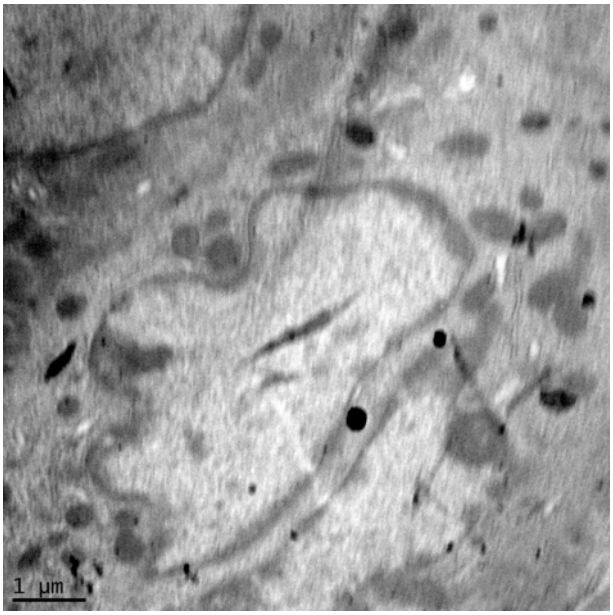


Figure 6: EM RAG of several columnar epithelial cells of a postnatal month 12 mouse labeled with ^{3}H-uridine, showing silver grains over the nuclei and mitochondria. x6,000.

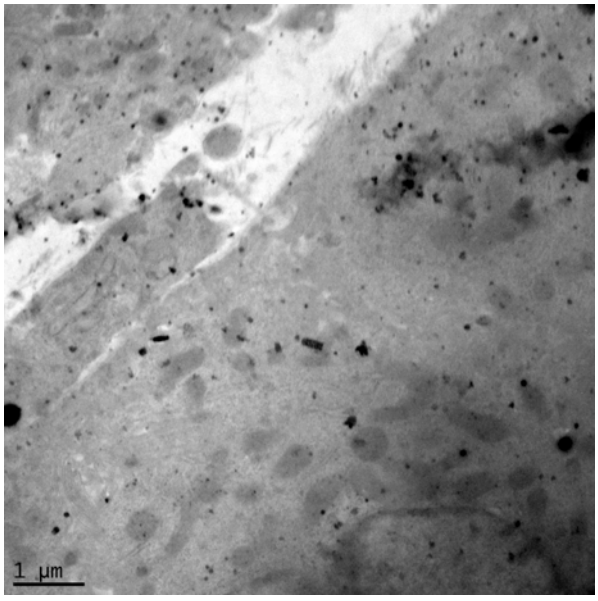
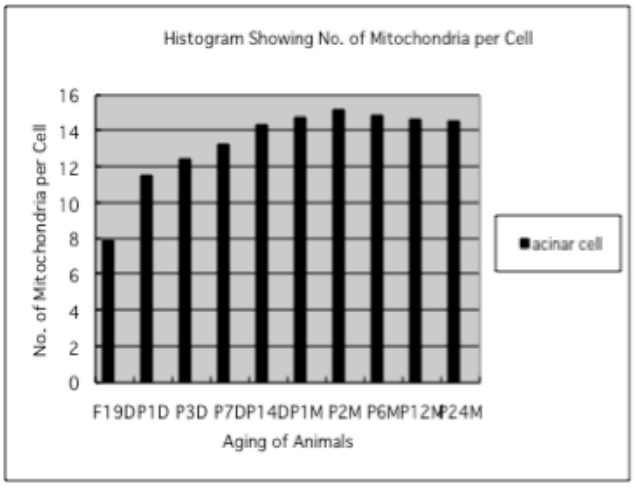


Figure 7: EM RAG of several columnar epithelial cells of a postnatal month 24 mouse labeled with ^{3}H-uridine, showing many silver grains over the nuclei and mitochondria. x6,000.

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. The results obtained from the number of mitochondria in columnar epithelial cells of respective animals in 10 aging groups at perinatal and newborn stages, from prenatal embryo day 19 to postnatal day 1, 3, 7, 14, and adult and senescent stages at month 1, 2, 6, 12, and 24, seemed to show an gradual increase from the prenatal day 19 to postnatal month 24.

Counting the number of mitochondria per cell at respective aging stages, it increased from prenatal embryo around 5.7/cell in average, to 6.2 at postnatal day 1, to 7.5 at day 3, to 9.3 at day 7, to 10.1 at day 14, to 11.7 at month 1, to 11.8 at month 2, then slightly

decreased to 11.2 at month 6, to 11.1 at month 12 and finally to 10.4 at month 24 as shown in Figure **8**. All the data from embryonic day 19 to postnatal month 24, were stochastically analyzed using variance and Student's t-test. The increases of mitochondrial numbers in the colonic columnar epithelial cells from embryonic day 19 to adult stage at postnatal month 2 were considered to be significant at P value <0.01. However, the slight decrease at the senescent stage from month 6 to 24 were considered to be not significant at P value <0.01.



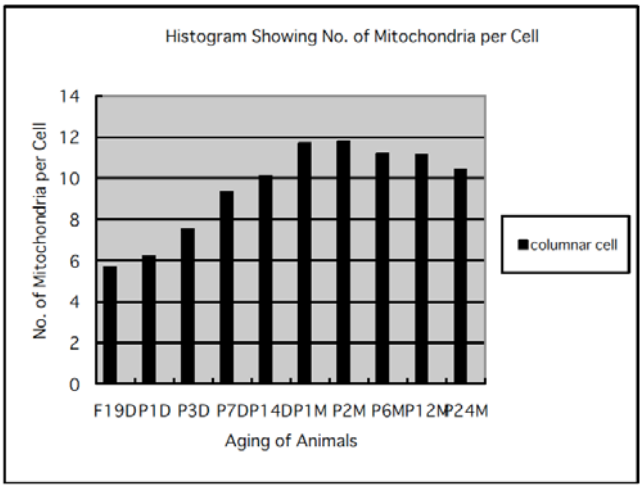
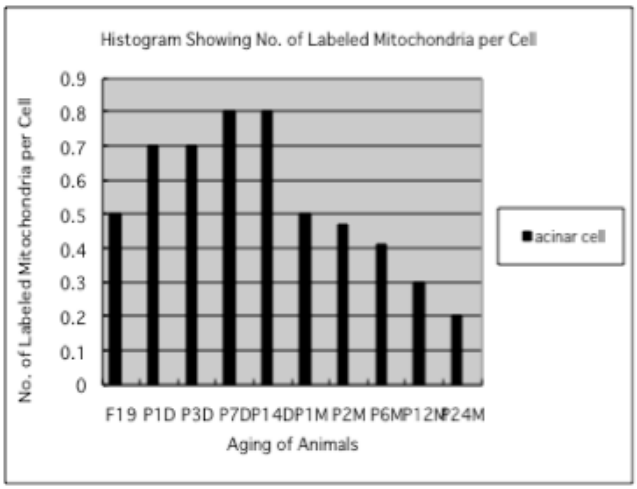


Figure 8: Histogram showing the numbers of mitochondria per cell in respective aging groups.

3.3.2. Mitochondrial RNA synthesis

The results of visual counting on the number of mitochondria labeled with silver grains obtained from 10 columnar epithelial cells of each animal labeled with ^{3}H-uridine demonstrating RNA synthesis in 10 aging groups at perinatal stages, from prenatal embryo day 19 (4.9/cell), postnatal day 1, 3, 7 and 14, to adult stages at month 1, 3, and 6, 12 and 24, increased gradually to day 1 (5.6), to day 3 (6.8) to day 7 (8.2) and day 14 (8.9), to month 1 (9.8), to month 2 (10.4), reaching the maximum, then decreased gradually to month 6 (10.2), to month 12 (10.3) and month 24 (9.8/cell) as shown in Figure **9**. The data were stochastically analyzed using variance and Student's t-test. The increases of the numbers of labeled mitochondria from embryo day 19 to postnatal month 2, were stochastically significant (P <0.01). However, the decreases from month 2 to month 24 were not significant.



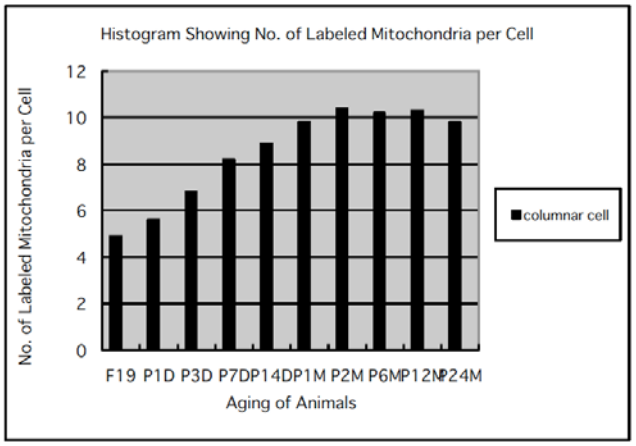


Figure 9: Histogram showing the numbers of labeled mitochondria per cell in respective aging groups labeled with ^{3}H-uridine.

3.3.3. The Labeling Index

Finally, the labeling indices of mitochondrial RNA synthesis in colonic columnar epithelial cells at respective aging stages were calculated from the number of labeled mitochondria (Figure **9**) dividing by the number of total mitochondria per cell (Figure **8**), which were plotted in Figure **10**.

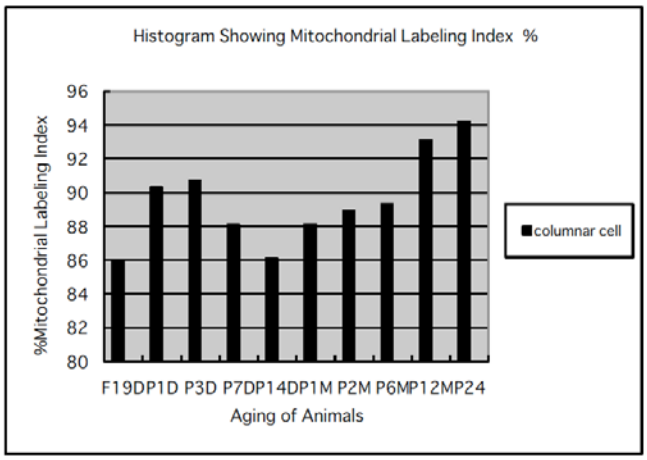


Figure 10: Histogram showing the average labeling indices in respective aging groups labeled with ^{3}H-uridine.

The results showed that the labeling indices increased from prenatal day 19 (85.9%) to postnatal newborn day 1 (90.3%), then increased to postnatal day 3 (90.7%) and gradually decreased to postnatal day 7 (88.1%), to day 14 (86.1%), to adult stages at month 1 (88.1%), then again increased to month 2 (86.1 %) and month 6 (89.3%), to month 12 (93.1%) and 24 (94.2%), reaching the maximum as shown in Figure **10**. From the results, the increase of the mitochondrial labeling indices in colonic columnar epithelial cells from embryo day 19 to newborn postnatal day 1 to 3 and decrease to postnatal day 7 and 14, increase again to month 2 and 24 were stochastically significant ($P < 0.01$).

4. DISCUSSION

From the results obtained in the present study on the colonic columnar epithelial cells of ddY aging mice at various ages in 10 groups from perinatal stages at embryo day 19, to newborn day 1, 3, juvenile day 7, 14, and young adult at postnatal month 1, 2, 6 as well as the senescent adult at postnatal month 12 and 24, it was shown that intramitochondrial RNA synthesis was observed in the colonic columnar epithelial cells of all the aging stages from prenatal embryos to postnatal newborn, juvenile and young adult, senescent adult stages and the number of mitochondria per cell showed increases due to aging, while the number of labeled mitochondria per cell and the labeling index showed increases and decreases due to aging. However, there was a discrepancy between the increases and decreases of the number of total mitochondria and labeled mitochondria which showed increases and decreases with the two peaks of increases in the labeling index. The inconsistency may be due to the difference of the timing when the mitochondria synthesized RNA at juvenile and young stages from postnatal day 1 to month 2 when the number of mitochondria increased rapidly. These results demonstrated that intramitochondrial RNA synthesis in the colonic epithelial cells revealed variations due to aging of individual animals depending upon the cellular activities at respective aging stages.

With regards 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 ^{3}H-thymidine demonstrate DNA synthesis [1, 4, 6, 10, 12-18]. The previous results obtained from the studies on the hepatocytes of aging mice by light and electron microscopic radioautography revealed that silver grains indicating DNA synthesis incorporating ^{3}H-thymidine were observed over the nuclei of some hepatocytes at perinatal stages from postnatal day 1 to day 14 and decreased due to aging [15-18]. Then, we lately observed the intramitochondrial DNA synthesis in the various organs such as the livers [12, 13, 19-22] adreno-cortical [14, 23-26], adreno-medullary cells [14, 27, 28] and the pancreatic acinar cells [92, 93], at various ages from fetal day 19 to postnatal newborn day 1, 3, juvenile day 7, 14 and to adult month 1, 2, 6, 12 and 24. In the present study, further data obtained from the colonic columnar epithelial cells from prenatal to adult senescent animals at postnatal month 12 and 24 were added.

On the other hand, we also studied the numbers of silver grains showing nuclear RNA synthesis resulting from the incorporations of ^{3}H-uridine into mitochondria indicating mitochondrial RNA synthesis demonstrated the silver grain localization over the mitochondria independently from the nuclei whether the nuclei were labeled with silver grains or not in the pancreatic acinar cells from prenatal embryo day 19 to postnatal month 24 during the development and aging [92, 93].

The numbers of labeled mitochondria showing RNA synthesis as well as the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 1 and day 3, reaching a maximum, then decreased to day 14, increased again from adult postnatal month 1, 2, 6 to the senescent stages at month 12 and 24, reaching another maximum.

With regards 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 [29-34]. Most of these authors observed DNA fibrils in mitochondria which were histochemically extracted by DN'ase. 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 [35]. It was calculated that such a single molecule had a molecular weight of about 10^7 daltons [36]. Mitochondria of various cells also contained a DNA polymerase, which was supposed to function in the replication of the mitochondrial DNA [37]. On the other hand, the incorporations of ^{3}H-thymidine into mitochondria demonstrating DNA synthesis were observed by means of electron microscopic radioautography in lower organism such as slime mold [38, 39], tetrahymena [40] or chicken fibroblasts in tissue culture under abnormal conditions [41]. However, these authors used old-fashioned developers consisting of methol and hydroquinone (MQ-developer) that 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 [7, 15], 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 [1, 12, 13, 42, 43]. These papers were the first which demonstrated intramitochondrial DNA synthesis incorporating ^{3}H-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 [8, 10, 44-46]. Those authors who used the M-Q developers maintained the resolution to be 100-160 nm [44, 45], while those authors who used the elon-ascorbic acid developer [8, 10, 46] 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 [1, 12, 13, 42, 43, 46]. For the analysis of electron radioautographs, Salpeter *et al*. [40] 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 [8, 10, 12, 13, 42, 43].

Then we also demonstrated intramitochondrial DNA synthesis incorporating ^{3}H-thymidine in some other established cell lines originated from human being such as HeLa cells [8, 10] or mitochondrial fractions prepared from *in vivo* mammalian cells such as rat and mouse [9, 11]. 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 [47], cultured rat sarcoma cells [48], mouse liver and pancreas cells *in vitro* [48, 50, 51], but also *in vivo* cells obtained from various organs such as the salivary glands [52], the livers [53-64,91], the pancreases [65], the tracheas [66], the lungs [67], the kidneys [68], the testis [69,70], the uterus [71,72], the adrenal glands [73-75], the brains [76], and the retina [77-81] 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 [8]. However, the relationship between the DNA synthesis and the aging of individual animals and men has not yet been fully clarified except a few papers published by Korr and associates on mouse brain [82-85]. They reported both nuclear DNA repair, measured as nuclear unscheduled DNA synthesis, and cytoplasmic DNA synthesis labeled with ^{3}H-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. They observed silver grains over cytoplasm of these cells by light microscopy and maintained that it was reasonable to interpret these labeling as ^{3}H-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 without 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 [53-64] 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.

Our previous studies [59, 60] also clarified that the DNA synthesis and cell proliferation by mitosis were the most active in the nuclei of mononucleate hepatocytes at the perinatal stages in contrast that binucleate cells were less active at the perinatal stage but the number of binucleate hepatocytes increased at senescent stages and the results suggest the possibility that the mitochondria in mononucleate hepatocytes synthesized their DNA by themselves which peaked at postnatal day 14 in accordance with the proliferation of mononucleate hepatocytes while binucleate hepatocytes increased after the perinatal stage and did not divide but remained binucleate keeping many mitochondria in their cytoplasm which were more in number than mononucleate hepatocytes at the senescent stage.

Thus, our previous papers were the first which dealt with the relationship between the DNA synthesis and aging in hepatocytes of mice *in vivo* at various ages by means of electron microscopic radioautography observing the small dot-like silver grains, due to incorporations of ^{3}H-thymidine, which exactly localized inside the mitochondria.

Later we also studied intramitochondrial DNA synthesis in adreno-cortical cells from prenatal day 19 to postnatal day 1, 3, 9, 14, month 1, 2, 6, 12 and 24 (year 2) and found that the numbers of mitochondria in 3 zones, glomerulosa, fasciculate and reticularis, increased reaching the maxima at postnatal month 2 and which kept continued until senescence up to 24 months (2 years). To the contrary, the numbers of labeled mitochondria and the labeling indices increased to postnatal month 2, reaching the maxima, then decreased to month 24 [23-28]. Later we also observed that the number of the pancreatic acinar cells showing DNA synthesis in mitochondria and the labeling indices increased to postnatal day 14, reaching the maxima, then decreased to month 24 [92]. On the other hand, we also demonstrated the results from the RNA synthesis in the livers and adrenal glands of aging mice which also revealed that an increase was observed by direct observation on mitochondria at electron microscopic level and obtaining accurate mitochondrial number and labeling indices in the hepatocytes and adreno-cortical and adreno-medullary cells. In the present study, we also demonstrated the RNA synthesis in the pancreatic acinar cells in 10 groups of developing and aging mice. There was a discrepancy between our results from the hepatocytes [59, 60], the adrenal cells [23-28], the pancreatic acinar cells [93] as well as the colonic columnar epithelial cells at present and the results from the several types of cells in the brains by Korr *et al*. [82-85]. The reason for this difference might be due to the difference between the cell types (hepatocytes, adrenal cells, pancreatic acinar cells or colonic columnar epithelial cells from our results and the brain cells from their results) or the difference between the observation by light or electron microscopy, i.e., direct observation of mitochondria by electron microscopy in our results or light microscopy, i.e., indirect observation of mitochondria without observing any mitochondria directly by Korr *et al*. [82-85].

Anyway, the results obtained from the colonic epithelial cells of aging mice at present should form a part of special cytochemistry [17] in cell biology, as well as a part of special radioautographology [12], i.e., the application of radioautography to the pancreas, as was recently reviewed by the present author including recent results dealing with various organs [86-93]. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future.

CONCLUSION

From the results obtained at present, it was concluded that almost all the columnar epithelial cells in the colons of mice at various ages, from prenatal embryo day 19 to postnatal newborn, day 1, 3, 7 and 14, and to postnatal month 1, 2, 6, 12 and 24, were labeled with silver grains showing RNA synthesis with ^{3}H-uridine in their mitochondria.

Quantitative analysis on the number of mitochondria in colonic columnar epithelial cells showed that they increased from prenatal embryo day 19 to postnatal day 1, 3, 7 to 14, continued to increase to month 1, month 2, reaching a maximum, then slightly decreased to month 6, 12 and finally to month 24. Likewise, the number of labeled mitochondria with ^{3}H-uridine showing RNA synthesis increased from prenatal day 19 to postnatal day 1, 3, 7, 14, to month 1 and 2, reaching a maximum, then slightly decreased to month 6, 12 and 24. To the contrary, the labeling index increased from prenatal day 19 to postnatal day 1 and 3, reaching a maximum, then decreased to day 7, 14, reaching a minimum, and increased again to month 1, 2, 6, 12 and 24.

These results demonstrated that the number of mitochondria in the colonic epithelial cells increased from perinatal stages to postnatal day 3, decreased to day 7 and 14, and again increased to month 2 and 6, keeping the maximum up to month 24, while the activity of mitochondrial RNA synthesis increased from prenatal to postnatal day 1 and 3, then decreased to postnatal day 14, and again increased to month 1, 2, 6, 12, 24, reaching the maximum due to aging of animals.

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REFERENCES

[1] Nagata T, Usuda N. Image processing of electron microscopic radioaautograms in clinical electron microscopy. J Clin Electron Microsc 1985; 18: 5-6.

[2] Nagata T, Usuda N, Ma H. Electron microscopic radioautography of nucleic acid synthesis in pancreatic acinar cells of prenatal and postnatal aging mice. Proc. 11th Internat. Cong. Electron Microsc, Kyoto, Japan, 1986; Vol. 3: pp. 2281-2282.

[3] Nagata T, Usuda N, Ma H. 1986; Application of high voltage electron microscopy to histochemistry of whole mount preparations of cultured cells or thick sections from embedded tissues. Proc. 11th Internat. Cong. Electron Micrsco. Kyoto, Japan, pp.1183-1184.

[4] Nagata T, Usuda N, Ma H. Electron microscopic radioautograpahy of nucleic acid synthesis in pancreatic acinar cells of prenatal and postnatal aging mice. Proc. 11th Internat. Cong. Electron Micrsco. Kyoto, Japan, 1986; pp. 2281-2282.

[5] Nagata T. Radiolabeling of soluble and insoluble compounds as demonstrated by light and electron microscopy. In, Recent Advances in Cellular and Molecular Biology. Vol. 6 Molecular Biology of Pyrimidines, DNA, peroxisomes, organelles and cell movements. Wegmann, R. J. and Wegmann, M. A. eds, Peeters Press, Leuven, Belgium 1992; pp. 9-21.

[6] Nagata T. Quantitative analysis of histochemical reactions: image analysis of light and electron microscopic radioautograms. Acta Histochem Cytochem 1993; 26: 281-91. http://dx.doi.org/10.1267/ahc.26.281

[7] Nagata T. Quantitative light and electron microscopic radioautographic studies on macromolecular synthesis in several organs of prenatal and postnatal aging mice. Chinese J Histochem Cytochem 1993; 2: 106-108.

[8] Nagata T. Radioautographic study on intramitochondiral nucleic acid synthesis: Its relationship to the cell cycle in cultivated cells. Proc. 4th Internat. Cong. Histochem. Cytochem. Kyoto, Japan 1972; 1: 223-224.

[9] Nagata T. Electron microscopic radioautography of intramitochondrial nucleic acid synthesis in mammalian cells *in vitro*. Proc. 8th Internat. Cong. Electron Microsc, Canberra, Australia 1974; 2: 346-47.

[10] Nagata T. Electron microscopic radioautography of RNA synthesis of HeLa cells in culture. Histochemie 1972; 32: 163-70. http://dx.doi.org/10.1007/BF00303731

[11] Nagata T, Iwadare N, Murata F. Mitochondiral and nucleolar RNA synthesis as revealed by electron microscopic radioatography. Proc. 5th Internat. Cong. Histochem. Cytochem. Bucharest, Romania 1976; 1: 242-243.

[12] Nagata T. Radioautographology, General and Special. In, Prog. Histochem. Cytochem, Graumann, W. ed, Urban-Fischer, Jena, Germany 2002; 32(2): pp. 57-228.

[13] Nagata T. Macromolecular synthesis in the livers of aging mice as revealed by electron microscopic radioautography. In, Prog. Histochem. Cytochem, Sasse, D, Schumacher, U, eds, Eslevier, Jena, Germany 2010; 45(1): pp. 1-80.

[14] Nagata T. Macromolecular synthesis in mitochondria in adrenal glands of mice as observed by electron microscopy. In, DNA Microarrays, Synthesis and Synthetic DNA, Nova Science Publishers, NY, USA 2011; in press.

[15] Nagata T, Shibata O, Nawa T. Incorporation of tritiated thymidine into mitochondrial DNA of the liver and kidney cells of chickens and mice in tissue culture. Histochemie 1967; 10: 305-308. http://dx.doi.org/10.1007/BF00304312

[16] Nagata T. Aging changes of macromolecular synthesis in various organ systems as observed by microscopic radioautogtraphy. Methods Find Exp Clin Pharmacol 1999; 21: 683-706. http://dx.doi.org/10.1358/mf.1999.21.10.795781

[17] Nagata T. Chapter 2, Special cytochemistry in cell biology. In, Jeon, K. W. ed, International Review of Cytology, Vol. 211, Academic Press, N. Y, USA 2001; pp. 33-154.

[18] Nagata T. Light and electron microscopic radioautographic studies on macromolecular synthesis in amitotic hepatocytes in aging mice. Cell Mol Biol 2003; 49: 591-11.

[19] Nagata T. Macromolecular synthesis in hepatocyte mitochondria of aging mice as revealed by electron microscopic radioautography. I: Nucleic acid synthesis. In, Modern Research and Educational Topics in Microscopy. Mendez-Vilas, A, Diaz, J. eds, Formatex Micrscopy Series No. 3, Formatex, Badajoz, Spain 2007; Vol. 1: pp. 245-258.

[20] Nagata T. Macromolecular synthesis in hepatocyte mitochondria of aging mice as revealed by electron microscopic radioautography. II: Protein synthesis. In,

Modern Research and Educational Topics in Microscopy. Mendez-Vilas, A. and Diaz, J. eds, Formatex Micrscopy Series No. 3, Formatex, Badajoz, Spain 2007; Vol. 1: pp. 259-271.

[21] Nagata T. Sexual difference between the macromolecular synthesis of hepatocyte mitochondria in male and female mice in aging as revealed by electron microscopic radioautography. Chapter 22. In, Women and Aging: New Research, H. T. Bennninghouse, A. D. Rosset, eds. Nova Biomed. Books, New York, USA 2009; pp. 461-487.

[22] Nagata T. Protein synthesis in hepatocytes of mice as revealed by electron microscopic radioautography. In, Protein Biosynthesis. Esterhouse, T. E. and Petrinos, L. B, eds, Nova Biomed. Books, New York, USA 2009; pp.133-161.

[23] Nagata T. Electron microscopic radioautographic study on mitochondrial DNA synthesis in adrenal cortical cells of developing mice. J Cell Tis Res 2008; 8: 1303-12.

[24] Nagata T. Electron microscopic radioautographic study on mitochondrial DNA synthesis in adrenal cortical cells of developing and aging mice. Sci World J 2008; 8: 683-97. http://dx.doi.org/10.1100/tsw.2008.93

[25] Nagata T. Electron microscopic radioautographic study on mitochondrial DNA synthesis in adreno-cortical cells of aging ddY mice. Bull Shinshu Inst Alternat Med Welfare 2009; 4: 51-66.

[26] Nagata T. Electron microscopic radioautographic study on mitochondrial RNA synthesis in adrenocortical cells of aging mice. Open Anat J 2010; 2: 91-97.

[27] Nagata T. Electron microscopic radioautographic study on mitochondrial DNA synthesis in adrenal medullary cells of developing and aging mice. J Cell Tissue Res 2009; 9: 1793-802.

[28] Nagata T. Electron microscopic radioautographic study on DNA synthesis of mitochondria in adrenal medullary cells of aging mice. Open Anat J 2009; 1: 14-24.

[29] Nass S, Nass MMK. Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. II. Enzymatic and other hydrolytic treatments. J Cell Biol 1963; 19: 593-29. http://dx.doi.org/10.1083/jcb.19.3.593

[30] Gibor A, Granick S. Plastids and mitochondria: Inheritable system. Science 1964; 145: 890-97. http://dx.doi.org/10.1126/science.145.3635.890

[31] Gahan PB, Chayen J. Cytoplasmic deoxyribonucleic acid. Internat Rev Cytol 1965; 18: 223-47. http://dx.doi.org/10.1016/S0074-7696(08)60555-0

[32] Nass MMK. The circularity of mitochondrial DNA. Proc Nat Acad Sci USA 1966; 56: 1215-22. http://dx.doi.org/10.1073/pnas.56.4.1215

[33] van Bruggen EFJ, Borst P, Ruttenberg GJCM, Gruber M, Kroon AM. Circular mitochondrial DNA. Biochim Biophys Acta 1966; 119: 437-39. http://dx.doi.org/10.1016/0005-2787(66)90210-3

[34] Sinclair JH, Stevens BJ. Circular DNA filaments from mouse mitochondria. Proc Natl Acad Sci USA 1966; 56: 508-14. http://dx.doi.org/10.1073/pnas.56.2.508

[35] Schatz G. Biogenesis of mitochondria. In, Membranes of Mitochondria and Chloroplasts. Racker E, ed, Van Nostrand-Reinhold, New York, USA 1970; pp. 251-314.

[36] Guttes E, Guttes S. Thymidine incorporation by mitochondria in Physarum polycephalum. Science 1964; 145: 1057-58. http://dx.doi.org/10.1126/science.145.3636.1057

[37] Schuster FL. A deoxyribose nucleic acid component in mitochondria of Didymium nigirpes, a slime mold. Exp Cell Res 1965; 39: 329-45. http://dx.doi.org/10.1016/0014-4827(65)90038-8

[38] Stone GE, Miller OL Jr. A stable mitochondrial DNA in Tetrahymena puriformis. Exp Zool 1965; 159: 33-37. http://dx.doi.org/10.1002/jez.1401590104

[39] Chévremont M. Cytoplasmic deoxyribonucleic acids: Their mitochondrial localization and synthesis in somatic cells under experimental conditions and during the normal cell cycle in relation to the preparation for mitosis. Cell Growth and Cell Division. Symposia of the Internat. Soc. for Cell Biol. Vol. 2, Harris R. J. C, ed, Academic Press, New York, USA 1963; p. 323-333.

[40] Salpeter MM, Bachmann L, Salpeter EE. Resolution in electron microscope radioautography. J Cell Biol 1969; 44: 1-20. http://dx.doi.org/10.1083/jcb.41.1.1

[41] Nadler NJ. The interpretation of grain counts in electron microscope radioautography. J Cell Biol 1971; 49: 377-82.

[42] Nagata T. Techniques and application of electron microscopic radioautography. J Electron Microsc 1996; 45: 258-74. http://dx.doi.org/10.1093/oxfordjournals.jmicro.a023442

[43] Nagata T. Techniques and application of microscopic radioautography. Histol Histopathol 1997; 12: 1091-24.

[44] Uchida K, Mizuhira V. Electron microscope autoradiography with special reference to the problem of resolution. Arch Histol Jap 1971; 31: 291-20. http://dx.doi.org/10.1679/aohc1950.31.291

[45] Murata F, Yoshida K, Ohno S, Nagata T. Electron microscopic radioautography using a combination of phenidon developer and domestic emulsion. Acta Histochem Cytochem 1979; 12: 443-50. http://dx.doi.org/10.1267/ahc.12.443

[46] Nagata T. Electron microscopic observation of target cells previously observed by phase-contrast microscopy: Electron microscopic radioautography of laser beam irradiated cultured cells. J Clin Electron Microsc 1984; 17: 589-90.

[47] Nagata T, Murata F, Yoshida K, Ohno S, Iwadare N. Whole mount radioautography of cultured cells as observed by high voltage electron microscopy. Proc. 5th Internat. Conf. High Voltage Electron Microsc. Kyoto, Japan 1977b; pp. 347-350.

[48] Nagata T, Iwadare N, Murata F. Electron microscopic radioautography of nucleic acid synthesis in cultured cells treated with several carcinogens. Acta Pharmacol Toxicol 1977; 41: 64-65.

[49] Nagata T, Murata F. Electron microscopic dry-mounting radioautography for diffusible compounds by means of ultracryotomy. Histochemistry 1977; 54: 75-82. http://dx.doi.org/10.1007/BF00493331

[50] Nagata T, Ohno S, Murata F. Electron microscopic dry-mounting radioautography for soluble compounds. Acta Pharmacol Toxicol 1977; 41: 62-63.

[51] Nagata T, Nawa T, Yokota S. A new technique for electron microscopic radioautography of soluble compouds. Histochemie 1969; 18: 211-49.

[52] Nagata T, Ito M, Chen S. Aging changes of DNA synthesis in the submandibular glands of mice as observed by light and electron microscopic radioautography. Ann Microsc 2000; 1: 13-22.

[53] Nagata T, Ohno S, Kawahara I, Yamabayashi S, Fujii Y, Murata F. Light and electron microscopic radioautography of nucleic acid synthesis in mitochondria and peroxisomes of rat hepatic cells during and after DEHP administration. Acta Histochem Cytochem 1979; 16: 610-11.

[54] Nagata T, Ohno S, Yoshida K, Murata F. Nucleic acid synthesis in proliferating peroxisomes of rat liver as revealed by electron microscopical radioautography. Histochem J 1982; 14: 197-204. http://dx.doi.org/10.1007/BF01041214

[55] Nagata T, Fujii Y, Usuda N. Demonstration of extranuclear nucleic acid synthesis in mammalian cells under experimental conditions by electron microscopic radioautography. Proc. 10th Internat. Cong. Electron Microsc, Hamburg, Germany 1982; Vol. 2: pp. 305-306.

[56] Ma H, Nagata T. Studies on DNA synthesis of aging mice by means of electron microscopic radioautography. J Clin Electron Microsc 1988; 21: 335-43.

[57] Ma H, Nagata T. Electron microscopic radioautographic study of DNA synthesis in the livers of aging mice. J Clin Electron Microsc 1988; 21: 715-16.

[58] Ma H, Gao F, Sun L, Jin C, Nagata T. Electron microscopic radioautographic study on the synthesis of DNA, RNA and protein in the livers of aging mice. Med Electron Microsc 1994; 27: 349-51.
http://dx.doi.org/10.1007/BF02349694

[59] Nagata T. Light and electron microscopic radioautographic studies on macromolecular synthesis in amitotic hepatocytes of aging mice. Cell Mol Biol 2003; 49: 591-11.

[60] Nagata T, Ma H. Electron microscopic radioautographic study on mitochondrial DNA synthesis in hepatocytes of aging mouse. Ann Microsc 2005; 5: 4-18.

[61] Nagata T. Electron microscopic radioautographic study on protein synthesis in hepatocyte mitochondria of developing mice. Ann Microsc 2006; 6: 42-54.

[62] Nagata T. Electron microscopic radioautographic study on macromolecular synthesis in hepatocyte mitochondria of aging mouse. J Cell Tissue Res 2007; 7: 1019-29.

[63] Nagata T. Electron microscopic radioautographic study on nucleic acids synthesis in hepatocyte mitochondria of developing mice. Trends Cell Mol Biol 2007; 2: 19-33.

[64] Nagata T. Electron microscopic radioautographic study on protein synthesis in mitochondria of binucleate hepatocytes in aging mice. Scientific World J 2007; 7: 1008-23.
http://dx.doi.org/10.1100/tsw.2007.140

[65] Nagata T, Usuda N, Ma H. Electron microscopic radioautography of nucleic acid synthesis in pancreatic acinar cells of prenatal and postnatal aging mice. Proc. 11th Internat. Cong. Electron Microsc, Kyoto, Japan 1986; Vol. 3, pp. 2281-2282.

[66] Sun L, Gao F, Jin C, Nagata T. DNA synthesis in the trachea of aging mice by light and electron microscopic radioautography. Acta Histochem Cytochem 1997; 30: 211-20.
http://dx.doi.org/10.1267/ahc.30.211

[67] Nagata T, Sun L. Electron Microscopic Radioautographic Study on Mitochondrial DNA and RNA Syntheses in Pulmonary Cells of Aging Mice. Ann Microsc 2007; 7: 36-59.

[68] Hanai T, Nagata T. Electron microscopic radioautographic study on nucleic acid synthesis in perinatal mouse kidney tissue. Med Electron Microsc 1994; 27: 355-57.
http://dx.doi.org/10.1007/BF02349695

[69] Gao F, Ma H, Sun L, Jin C, Nagata T. Electron microscopic radioautographic study on the nucleic acid and protein synthesis in the aging mouse testis. Med Electron Microsc 1994; 27: 360-62.
http://dx.doi.org/10.1007/BF02349697

[70] Gao F, Chen S, Sun L, Kang W, Wang Z, Nagata T. Radioautographic study of the macromolecular synthesis of Leydig cells in aging mouse testis. Cell Mol Biol 1995; 41: 145-50.

[71] Yamada AT, Nagata T. Light and electron microscopic radioautography of DNA synthesis in the endometria of pregnant ovariectomized mice during activation of implantation window. Cell Mol Biol 1992; 38: 763-74.

[72] Yamada AT, Nagata T. Light and electron microscopic radioautographic studies on the RNA synthesis of peri-implanting pregnant mouse uterus during activation of receptivity for blastocyst implantation. Cell Mol Biol 1993; 39: 221-33.

[73] Ito M, Nagata T. Electron microscopic radioautographic study on DNA synthesis and the ultrastructure of the adrenal gland in aging mice. Med Electron Microsc 1996; 29: 145-52.
http://dx.doi.org/10.1007/BF02348068

[74] Ito M. Radioautographic studies on aging changes of DNA synthesis and the ultrastructural development of mouse adrenal gland. Cell Mol Biol 1996; 42: 279-92.

[75] Nagata T. Electron microscopic radioautographic study on mitochondrial DNA synthesis in adrenal cortical cells of developing mice. J Cell Tis Res 2008; 8: 1303-12.

[76] Cui H, Gao F, Ma H, Nagata T. Study on DNA synthesis of cellular elements in the cerebella of aging mice by light and electron microscopic radioautography. Proc. 4th China-Japan Joint Histochem. Cytochem. Symp, Chongqing Publishing House, Chongqing, China 1996; pp. 111-112.

[77] Gunarso W. Radioautographic studies on the nucleic acid synthesis in the retina of chicken embryo II. Electron microscopic radioautography. Shinshu Med J 1984; 32: 241-48.

[78] Gunarso W, Gao F, Cui H, Ma H, Nagata T. A light and electron microscopic radioautographic study on RNA synthesis in the retina of chick embryo. Acta Histochem 1996; 98: 300-22.
http://dx.doi.org/10.1016/S0065-1281(96)80024-9

[79] Gunarso W, Gao F, Nagata T. Development and DNA synthesis in the retina of chick embryo observed by light and electron microscopic radioautography. Cell Mol Biol 1997; 43: 189-201.

[80] Kong Y, Nagata T. Electron microscopic radioautographic study on nucleic acid synthesis of perinatal mouse retina. Med Electron Microsc 1994; 27: 366-68.
http://dx.doi.org/10.1007/BF02349699

[81] Nagata T. Aging changes of macromolecular synthesis in the avian and mammalian eyes as revealed by microscopic radioautography. Ann Rev Biomed Sci 2006; 8: 33-67.

[82] Korr H, Phillipi V, Helg C, Schiefer J, Graeber MB, Kreutzberg GW. Unscheduled DNA synthesis and mitochondrial DNA synthetic rate following injuring of the facial nerve. Acta Neuropathol 1997; 94: 557-66.
http://dx.doi.org/10.1007/s004010050750

[83] Korr H, Kurz C, Seidler TO, Sommer D, Schmitz C. Mitochondrial DNA synthesis studied autoradiographically in various cell types *in vivo*. Braz J Med Biol Res 1998; 31: 289-98.
http://dx.doi.org/10.1590/S0100-879X1998000200012

[84] Schmitz C, Axmacher B, Zunker U, Korr H. Age related changes of DNA repair and mitochondrial DNA synthesis in the mouse brain. Acta Neuropathol 1999; 97: 71-81.
http://dx.doi.org/10.1007/s004010050957

[85] Schmitz C, Materne S, Korr H. Cell-type-specific differences in age-related changes of DNA repair in the mouse brain - Molecular basis for a new approach to understand the selective neuronal vulnerability in Alzheimer's disease. J Alzheimer's Disease 1999; 1: 387-407.

[86] Nagata T. Recent studies on macromolecular synthesis labeled with ^{3}H-thymidine in various organs as revealed by electron microscopic radioautography. Curr Radiopharm 2009; 2: 118-28.
http://dx.doi.org/10.2174/1874471010902020118

[87] Nagata T. Electron microscopic radioatuographic studies on macromolecular synthesis in mitochondria of various cells. 18EMSM Conference Proc. 9th Asia-Pacific Microscopy Conference (APMC9), Kuala Lumpur, Malaysia 2009; pp. 48-50.

[88] Nagata T. Electron microscopic radioautographic studies on macromolecular synthesis in mitochondria of animal cells in aging. Ann Rev Biomed Sci 2009; 11: 1-17.

[89] Nagata T. Electron microscopic radioautographic studies on macromoleclular synthesis in mitochondria of some organs in aging animals. Bull Shinshu Inst Alternat Med Welfare 2009; 4: 15-38.

[90] Nagata T. Electron microscopic radioautographic studies on macromolecular synthesis in mitochondria ofanimal cells in aging. Ann Rev Biomed Sci 2010; 12: 1-29.

[91] Nagata T. Macromolecular synthesis in the livers of aging mice as revealed by electron microscopic radioautography. In, Progress in Histochemistry and Cytochemistry. Vol. 45, No. 1, pp. 1-80, Elsevier, Jena, New York, 2010

[92] Nagata T. DNA synthesis in the pancreatic acinar cells of aging mice as revealed by electron microscopic radioautography. IIOABJ (Institute of Integrative Omics and Applied Biotechnology) 2011; 2: 31-39.

[93] Nagata T. RNA Synthesis in the pancreatic acinar cells of aging mice as revealedby electron microscopic radioautography. Curr Radiophamaceutics 2012; 5: 5-14. http://dx.doi.org/10.2174/1874471011205010005

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