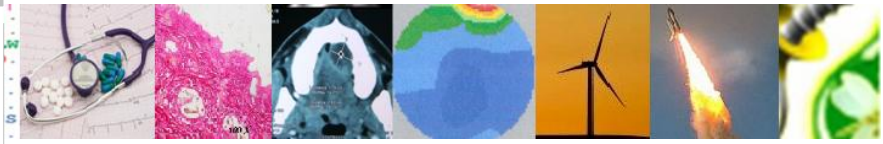

ISSN: 0976-3104

*RESEARCH: MOLECULAR BIOLOGY*

# DNA SYNTHESIS IN THE PANCREATIC ACINAR CELLS OF AGING MICE AS REVEALED BY ELECTRON MICROSCOPIC RADIOAUTOGRAPHY

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**Received on:** 15th-Feb-2011; **Revised on:** 18th-Mar-2011; **Accepted on:** 18th-Mar-2011; **Published on:** 14th-May-2011

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## ABSTRACT

***For the purpose of studying the aging changes of macromolecular synthesis in animal cells, we studied 10 groups of aging mice during development and aging from fetal day 19 to postnatal month 24. They were injected with $^{3}$H-thymidine, a precursor for DNA synthesis, sacrificed and the pancreatic tissues were taken out, fixed and processed for light and electron microscopic radioautography. On many radioautograms the localization of silver grains demonstrating DNA synthesis incorporating $^{3}$H-thymidine in the pancreatic acinar cells in respective aging groups were analyzed. The number of silver grains and the number of cell organelles in each cell in respective aging groups were analyzed quantitatively in relation to the aging of animals. The results revealed that the DNA synthesis as expressed by the number of silver grains in cell nuclei, cell organelles, changed with the aging of animals. The number of mitochondria, the number of labeled mitochondria and the mitochondrial labeling index labeled with silver grains were counted in each pancreatic acinar cell. It was demonstrated that the number of mitochondria increased from embryonic day 19 to postnatal newborn day 1, 3, 9, 14, adult month 1, 2 and 6, reaching the maxima, then decreased to senile year 1 to 2.. On the other hand, the number of labeled mitochondria and the labeling indices showing DNA synthesis at various ages increased from embryonic day 19 to postnatal newborn day 1, 3, 9, 14, reaching the maxima and decreased to adult month 1, 2 and 6, to senile year 1 to 2, indicating the aging changes. These results demonstrated that intramitochondrial DNA synthesis in the pancreatic acinar cells increased and decreased 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.***

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**Keywords:** *DNA synthesis; mitochondria; pancreatic acinar cells; aging; mice*

## [I] INTRODUCTION

The pancreas is a large gland in animals and men, next to the liver, among the digestive glands connected to the intestines. It consists of exocrine and endocrine portions and takes the shape of a compound acinous gland. The exocrine portion is composed of ductal epithelial cells, centro-acinar cells, acinar cells and connective tissue cells, while the endocrine portion, designated as the islet of Langerhans, is composed of 3 types of endocrine cells, A, B, C cells and connective tissue cells.

We have studied the macromolecular synthesis of the aging mouse pancreas at various ages by means of light and electron microscopic radioautography. We first studied the DNA synthesis of mouse pancreas by LM and EM RAG using $^{3}$H-thymidine [1, 2, 3]. Light and electron microscopic radioautograms (LM and EM RAG) of the pancreas revealed that the nuclei of pancreatic acinar cells, centro-acinar cells, ductal epithelial cells, and endocrine cells were labeled with $^{3}$H-thymidine demonstrating DNA synthesis **[Figures–1-10]**. The number of labeled cells were counted and the labeling indices of these cells in 10 groups of litter mate mice, fetal day 19, postnatal day 1, 3, 7, 14, and month 1, 6, 12 (year 1) and 24 (2 years) were analyzed. The labeling indices of these cells

ISSN: 0976-3104

reached the maxima at day 1 after birth and decreased gradually from postnatsl day 1 **[Figure–1]** to day 7 **[Figure–2],** day 14 **[Figures– 3-5]**, month 1 **[Figure-6],** month 2 **[Figure–7]**, month 6 and month 24 or 2 years **[Figure-10]**. The maximum of the labeling indices in the acinar cells proceeded to the ductal and centro-acinar cells, suggesting that the acinar cells completed their development earlier than the ductal and centro-acinar cells [1, 2, 3].

On the other hand, LM and EM RAG of pancreas of mouse injected with $^{3}$H-uridine into the aging mice from embryo to postnatal year 2, demonstrated its incorporation into exocrine and then in endocrine cells, and more in pancreatic acinar cells than in ductal or centro-acinar cells [1, 2, 3]. Among the acinar cells, the number of silver grains increased after birth to day 7 and day 14, then decreased to month 1, 2, 6, 12, and 24 with aging. Quantification of silver grains in the nucleoli, chromatin, and cell body were carried out by X-ray microanalysis [5, 6], which verified the results obtained by visual grain counting. In EM RAG obtained from the pancreas of fetal day 19 embryos, newborn day 1 and newborn day 14 mice labeled with $^{3}$H-uridine, demonstrating RNA synthesis, the number of silver grains in the nucleoli, nuclear chromatin and cytoplasm increased [6, 7]. In order to quantify the silver contents of grains observed over the nucleoli, nuclei and cytoplasm, X-ray spectra were recorded by energy dispersive X-ray microanalysis (JEM-4000EX TN5400), demonstrating Ag-Kα peaks at higher energies.

On the other hand, in contrast to the DNA and RNA syntheses in nuclei and nucleoli in various cells of aging mice, we also found the silver grains due to DNA and RNA synthesis in mitochondria of various isolated cells such as the livers and kidneys in vitro showing intramitochondrial DNA and RNA syntheses [8]. We later found that the activities of DNA and RNA syntheses in mitochondria of various cells changed due to aging of individual animals [8, 9, 10, 11].

Thus, we have recently concentrated to clarify the intramitochondiral DNA and RNA as well as protein synthesis in various cells of aging mice [12], especially in hepatocytes which contained many mitochondria [13]. This paper deals with the intramitochondrial DNA synthesis in pancreatic acinar cells of aging ddY mice at various ages in 10 groups from prenatal embryos to postnatal 2 years at senescence.

## [II] MATERIALS AND METHODS

### 2.1. The experimental animals

The pancreatic 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-thymidine, a DNA precursor, and the pancreatic 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 as well as the principles of laboratory animal care in NIH publication No. 86-23 (revised 1985).

### 2.2. Procedures of electron microscopic radioautography

All the animals were injected intraperitoneally with $^{3}$H-4-thymidine (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 right end of the pancreatic gland was taken out from each animal, excised and 3 small pieces of the pancreatic tissues (size 1mm x 1mm x 1mm) 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 electron microscopic radioautography, semithin sections at 0.2µm thickness, thicker than conventional ultrathin sections in order to shorten the exposure time, 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, 6, 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 electron micrographs

For quantitative analysis of electron micrographs, twenty EM radioautograms showing cross sections of pancreatic acinar cells from each group, based on the electron microscopic photographs taken after observation on at least 100 pancreatic acinar 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.

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## [III] RESULTS

### 3.1. Morphological observations

The pancreatic tissues obtained from ddY strain mice at various ages from embryo day 19 to postnatal month 24, consisted of 2 portions, the exocrine portion **[Figures–1–4, 6–10]** and the endocrine portion or designated as the islets of Langerhans **[Figure– 5]**. The exocrine portion is consisted of several cell types, the pancreatic acinar cells **[Figures–1-3, 6-10]**, the centroacinar cells **[Figure– 4]**, ductal cells and fibroblasts, as observed by electron microscopy. The acinar cells are main components of the exocrine portions which contained well developed endoplasmic reticulum, zymogen granules, and many mitochondria in the cytoplasm. Because the number of mitochondria in the pancreatic acinar cells were relatively much more than the other cells, only the pancreatic acinar cells were analyzed in this study.

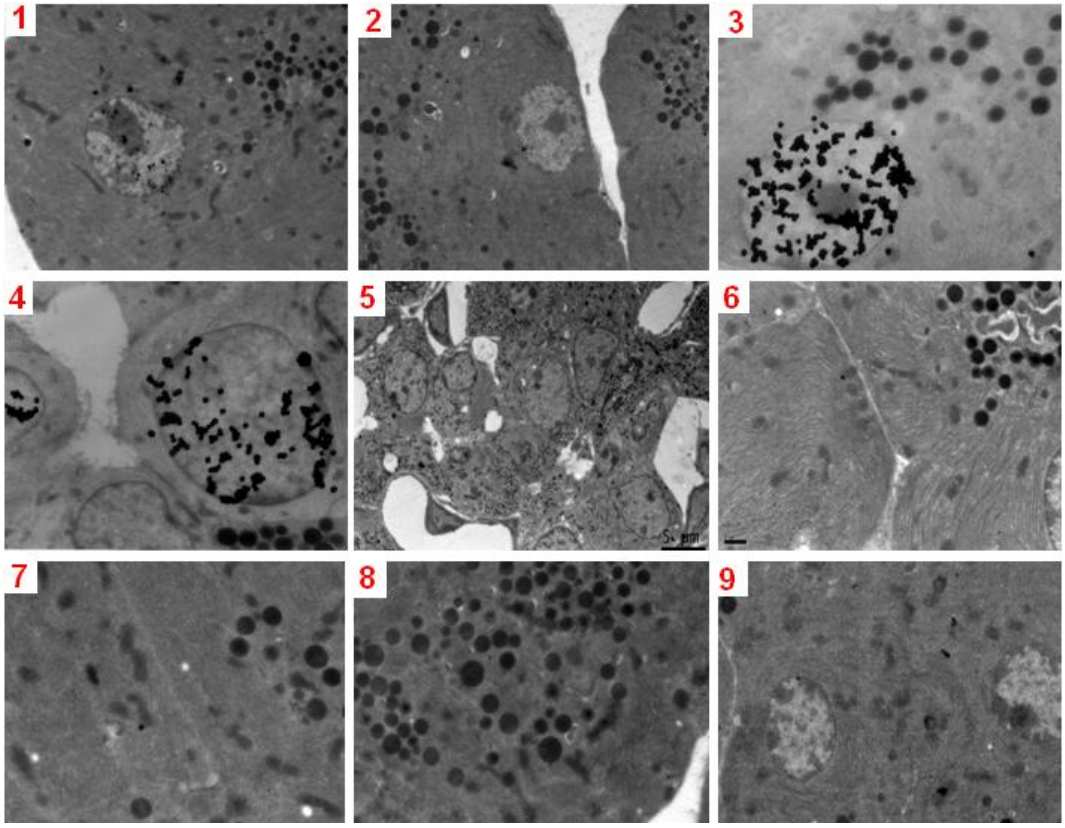


**Fig: 1.** EM RAG of a pancreatic acinar cell of a postnatal day 1 mouse labeled with $^{3}$H-thymidine. x3,000 **Fig: 2.** EM RAG of 2 pancreatic acinar cells of a postnatal day 7 mouse labeled with $^{3}$H-thymidine. x3,000 **Fig: 3.** EM RAG of a pancreatic acinar cell of a postnatal day 14 mouse labeled with $^{3}$H-thymidine. x5,000 **Fig: 4.** EM RAG of 2 pancreatic centro-acinar cells of a postnatal day 14 mouse labeled with $^{3}$H-thymidine. x5,000 **Fig: 5.** EM RAG of endocrine portion of a postnatal day 14 mouse labeled with $^{3}$H-thymidine. x3,000 **Fig: 6.** EM RAG of 3 pancreatic acinar cells of a postnatal month 1 mouse labeled with $^{3}$H-thymidine. x3,000 **Fig: 7.** EM RAG of a pancreatic acinar cell of a postnatal month 2 mouse labeled with $^{3}$H-thymidine. x5,000 **Fig: 8**. EM RAG of a pancreatic acinar cell of a postnatal month 6 mouse labeled with $^{3}$H-thymidine. x5,000 **Fig: 9.** EM RAG of 2 pancreatic acinar cells of a postnatal month 12 mouse labeled with $^{3}$H-thymidine. x3,000

### 3.2. Radioautographic observations

Observing electron microscopic radioautograms, the silver grains were found over the nuclei of some pancreatic acinar cells **[Figures–1, 3, 4]**, labeled with $^{3}$H-thymidine, demonstrating DNA synthesis mainly in perinatal stages at embryonic day 19, postnatal day 1 **[Figure–1]** and day 3, increased to day 7 and day 14 **[Figures–3, 4]**, reaching the maximum showing labeled mitochondria around 0.8-0.9/cell, then decreased to adult stage at month 1, month 2 and 6, showing labeled mitochondria around 0.4-0.5/cell.

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]

ISSN: 0976-3104

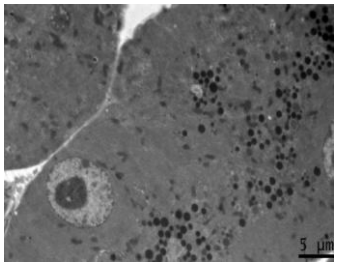

**Fig: 10. EM RAG of 3 pancreatic acinar cells of a postnatal month 24 mouse labeled with $^{3}$H-thymidine. x3,000**

### *3.3.2. Mitochondrial DNA synthesis*

The results of visual counting on the number of mitochondria labeled with silver grains obtained from 10 pancreatic acinar cells of each animal labeled with $^{3}$H-thymidine demonstrating DNA synthesis in 10 aging groups at perinatal stages, from prenatal embryo day 19 (0.5/cell), postnatal day 1, 3, 7 and 14, to adult stages at month 1, 3, and 6, 12 and 24, increased gradually to day 7 and 14, reaching the maximum (0.8/cell), then decreased gradually to month 1, 2, 6, 12 and 24 (0.2/cell) as shown in **Figure– 12**. 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 day 14, as well as the decreases from day 14 to month 24 were stochastically significant (P <0.01).

### *3.3.3. The labeling index*

Finally, the labeling indices of mitochondrial DNA synthesis in pancreatic acinar cells at respective aging stages were calculated from the number of labeled mitochondria **[Figure–11]** dividing by the number of total mitochondria per cell **[Figure– 10]** which were plotted in **Figure–13**.

The results showed that the labeling indices gradually increased from prenatal day 19 (6.4%) to postnatal newborn day 1 (6.1%), day 3 (5.6%), day 7 (6.1%), day 14 (5.6%), reaching the maximum, and decreased to adult stages at month 1 (3.4%), month 2 (3.1%), month 6 (2.8%), month 12 (2.1%) and 24 (1.4%) as shown in **Figure–13**. From the results, the increases of the mitochondrial labeling indices in pancreatic cells from embryo day 19 to postnatal day 14, as well as the decreases from day 14 to month 24 were stochastically significant (P <0.01).

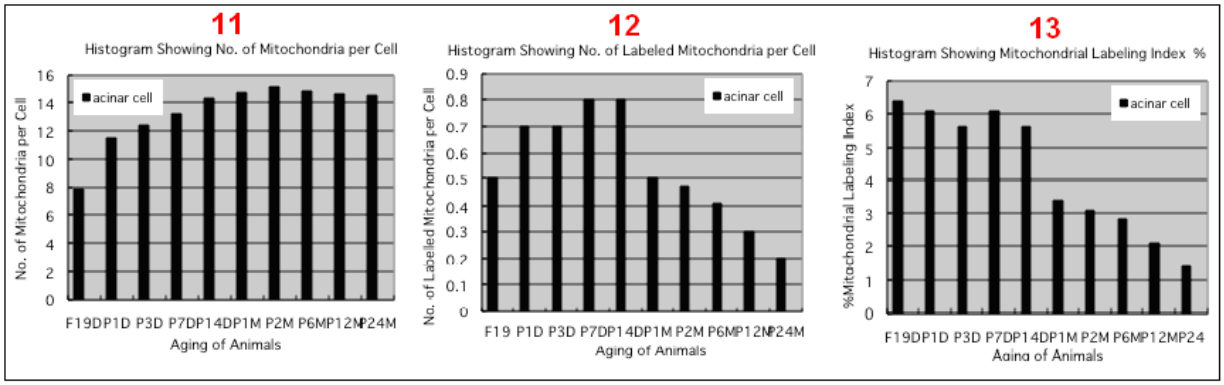


**Fig: 11.** Histogram showing average number of mitochondria per cell in a pancreatic acinar cell of aging mice at various ages from prenatal day 19 to postnatal month 24 **Fig: 12.** Histogram showing average number of labeled mitochondria per cell in a pancreatic acinar cell of aging mice at various ages from prenatal day 19 to postnatal month 24, labeled with $^{3}$H-thymidine **Fig: 13.** Histogram showing labeling indices of labeled mitochondria per cell in aging mice at various ages from prenatal day 19 to postnatal month 24, labeled with $^{3}$H-thymidine

## [IV] DISCUSSION

From the results obtained in the present study on the pancreatic acinar cells of ddY aging mice at various ages in 10 groups from perinatal stages at embryo day 19, to newborn day 1, 3, 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 DNA synthesis was observed in the pancreatic acinar cells of all the aging stages from prenatal embryos to postnatal newborn, young juvenile and 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 indices showed increases and decreases due to aging. These results demonstrated that intramitochondrial DNA synthesis in the pancreatic acinar cells revealed variations due to aging of individual animals depending upon the cellular activities at respective aging stages.

With regards to 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

ISSN: 0976-3104

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] and adreno-medullary cells [14, 27, 28], at various ages from fetal day 19 to postnatal newborn day 1, 3, 7, juvenile day 14 and to adult month 1, 2, 12 and 24. In the present study, further data obtained from the pancreatic acinar cells from prenatal to adult senescent animals at postnatal month 12 and 24 were added.

From these studies, the numbers of silver grains showing nuclear DNA synthesis resulting from the incorporations of $^{3}$H-thymidine into mitochondria indicating mitochondrial DNA 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. The numbers of labeled mitochondria showing DNA synthesis as well as the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 14, reaching the maxima, and then decreased to the adult stages at month 1, 2, 6, 12 and 24.

With regards to DNA in mitochondria in animal cells or plastids in plant cells, many studies have been reported in various cells of various plants and animals since 1960s [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) which produced coarse spiral silver grains resulting in inaccurate localization over cell organelles when observed by electron microscopy. All of these authors showed photographs of electron radioautographs with large spiral-formed silver grains (2-3 µm in diameter) localizing not only over the mitochondria but also outside the mitochondria. In order to obtain smaller silver grains, we first used elon-ascorbic acid developer after gold latensification [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 liver [53-64], the pancreas [65], the trachea [66], the lung [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, and epithelial cells as observed by light microscopic radioautography using paraffin sections. They observed silver

ISSN: 0976-3104

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].

The present results 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 pancreatic acinar cells in 10 groups of developing and aging mice. There was a discrepancy between our results from the hepatocytes [59, 60] and the adrenal cells [23-28] as well as the pancreatic acinar 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 cells from our results and the brain cells from their results) or the difference between the observation by electron microscopy, i.e., direct observation of mitochondria 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 pancreartic acinar 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 [86-90]. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future.

## [V] CONCLUSION

From the results obtained at present, it was concluded that almost all the pancreatic acinar cells in the pancreatic exocrine portions 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 DNA synthesis with $^{3}$H-thymidine in their mitochondria. Quantitative analysis on the number of mitochondria in pancreatic acinar cells resulted in increases from the prenatal day to postnatal day 1, 3, 9, 14, to month 1 and 2, reaching the maximum at postnatal month 2, then slightly decreased to month 6, 12 and 24. To the contrary, the numbers of labeled mitochondria with $^{3}$H-thymidine showing DNA synthesis and the labeling indices also increased from prenatal day 19 to postnatal day 14, reaching the maximum at postnatal day 14, and then decreased to month 1, 2, 6, 12 and 24. These results demonstrated that the number of mitochondria in pancreatic acinar cells increased from perinatal stages to postnatal month 2, keeping the maximum up to month 24, while the activity of mitochondrial DNA synthesis increased to postnatal day 14, reaching the maximum, then decreased to month 24 due to aging of animals.

## ACKNOWLEDGEMENT

This study was carried out after the author retired from Shinshu University School of Medicine and was not supported by any research grant. The author thanks Dr. Kiyokazu Kametani, Technical Official, Research Center for Instrumental Analysis, Shinshu University, for his technical assistance during the course of this study.

ISSN: 0976-3104

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